Electronic Supplementary Material (ESI) for Chemical Communications
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Supplementary Information

First synthetic analogues of diphosphoinositol polyphosphates: interaction with PP-InsP$_5$ kinase

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Table of Contents

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Enzyme Assays. Due to the reversibility of inositol pyrophosphate kinases,[1] the dephosphorylation of 1,5-$[PP]_2$-$InsP_4$ by 2.5 μg mL^{-1} $hPPIP5K2^{KD}$ (residues 1-366) was assayed for some experiments: The enzyme was incubated at 24 °C for 30 min with buffer containing 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.1 mM ADP and 0.1 μM 1,5-$[PP]_2$-$InsP_4$ in a 20 μL assay. The generated ATP was measured by luciferase assay (Invitrogen Catalog number A22066). In other experiments, the reactions were assayed in the forward (ATP-consuming) direction. Either $InsP_6$ or 5-PA-$InsP_5$ (40 μM) was incubated with 20 μg mL^{-1} $hPPIP5K2^{KD}$ (residues 1–366) for the indicated times at 37 °C in 20 mM Hepes-NaOH pH 7.2, 50 mM KCl, 1 mg mL^{-1} bovine serum albumen, 1 mM EDTA disodium salt, 1mM ATP disodium salt, 3 mM $MgSO_4$, 1 mM DTT, 20 μg mL^{-1} $hPPIP5K2^{KD}$ in a 250 μL reaction. Following quenching at 100 °C for 3 min, reactions were cooled on ice and then centrifuged at 4 °C, 15,000 × g for 5 min. Supernatants were concentrated in a SpeedVac to approximately 50 μL, then loaded onto a pre-run 16 cm PAGE gel with Orange G loading dye. After electrophoresis, reaction products were stained with Toluidine Blue.[2] Each lane contained 10 nmol of $InsP_6$ or 5-PA-$InsP_5$.

Protein Expression, Purification, Crystallization and Structure Determination. The catalytic domain of hPPIP5K2 (residues 1–366 and 41–366) was sub-cloned, expressed and purified as before.[3] The catalytic domain of PPIP5K2 (residues 41–366) was crystallized by hanging drop vapor diffusion against a well buffer of 12% (w/v) PEG 3350, 20 mM $MgCl_2$, 0.1 M HEPES, pH 7.0, 1 mM AMPPNP and 2 mM $CdCl_2$ at 4 °C. The crystals were then soaked with 2 mM compound **2** in a stabilizing buffer containing 22% (w/v) PEG 3350, 10 mM $MgCl_2$, 0.1 M sodium acetate, pH 5.2 at 4 °C for 3 days. Cryosolvent was prepared by adding 33% ethylene glycol into the soaking buffer. Diffraction data were collected using APS beamlines 22-ID. All data

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were processed with the program HKL2000.[4] The structure was determined using rigid body and direct Fourier synthesis, and refined with the equivalent and expanded test sets. The structure was further manually rebuilt with COOT[5] and refined with PHENIX[6] and REFMAC[7] from the CCP4 package. Ligand topology and parameter files were prepared using the PRODRG server.[8] The molecular graphics representations were prepared with the program PyMol (Schrödinger, LLC). Atomic coordinates and structure factors have been deposited with the Protein Data Bank with accession code 4GB4.

Data collection and structure refinement statistics

Data collection	
Space group	$P2_12_12_1$
Cell dimensions (a,b, c (Å))	110.8, 41.5, 89.7
Resolution (Å)*	50-1.9 (1.93)
Rsym *	0.062 (0.386)
I/σI*	24.6 (3.4)
Completeness (%)*	98.3(100.0)
Redundancy *	5.6
Refinement	
Resolution(Å)*	33.23-1.9 (1.95)
No. reflections	30869
R_{work} *	19.2 (20.6)
R_{free} *	22.2 (26.4)
No. atoms	
Protein	2692
Ligand/ion	74
Solvent	330
B-factors (Å2)	
Protein	28.8
Ligand/ion	43.0
Solvent	41.3
R.m.s. deviations	
Bond length(Å)	0.01
Bond Angle (°)	1.23

* The numbers in parentheses are for the highest resolution shell.

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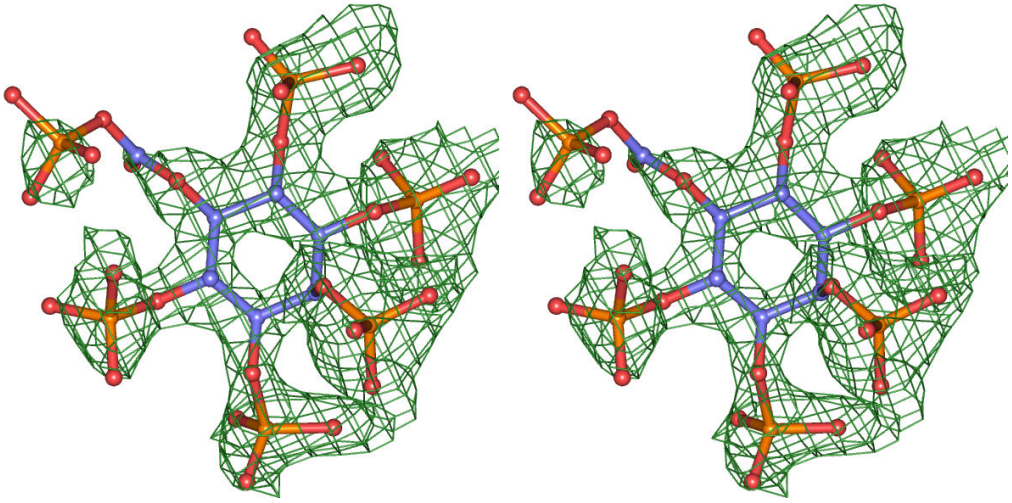
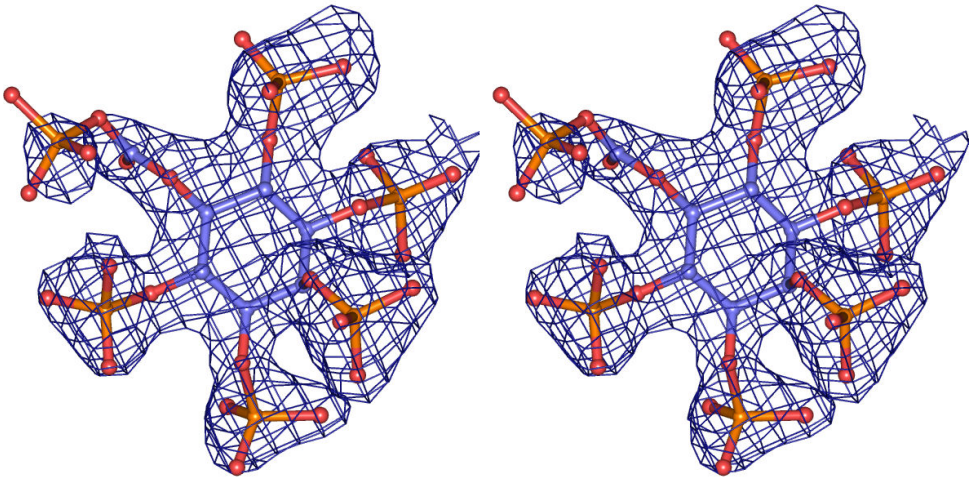
Fo-Fc



2Fo-Fc



Electron density maps. Stereo view of simulated-annealing omit difference map (F_o-F_c) (green) is contoured at 2.5 σ and $2F_o$-F_c map (blue, contoured at 1.3 σ).

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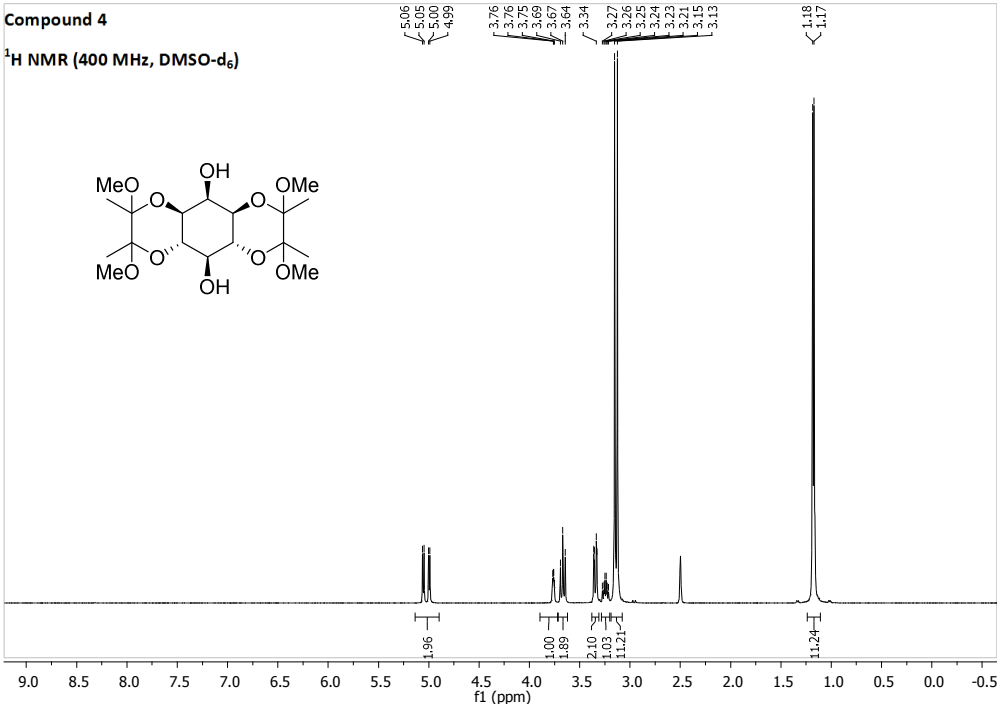
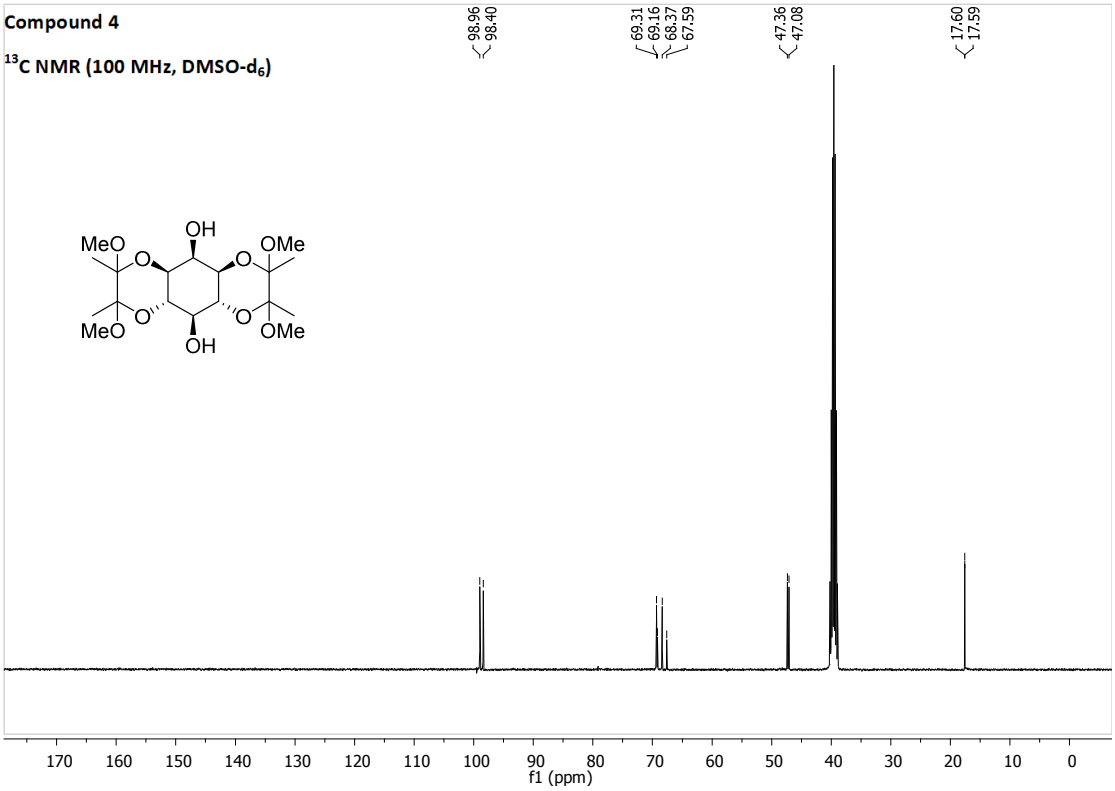
General Chemistry Methods

Chemicals were purchased from Sigma-Aldrich, Acros, Alfa Aesar or Fluka and used without further purification. Anhydrous solvents from Sigma-Aldrich were used without further treatment. TLC was performed on precoated plates (Merck Aluminum sheets silica 60 F_{254}, art No. 5554). Chromatograms were visualised under UV light and by dipping plates into either phosphomolybdic acid in EtOH or alkaline $KMnO_4$ solution, followed by heating. Ion exchange chromatography was performed on an LKB-Pharmacia Gradifrac medium pressure ion-exchange chromatograph using Q Sepharose Fast Flow resin and a gradient of 0 to 100% 2.0 $moldm^{-3}$ aqueous triethylammonium bicarbonate (TEAB). Proton ^{1}H NMR, COSY, HMBC and HMQC spectra were recorded on Bruker Avance III (400 MHz and 500 MHz) spectrometers. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.0 ppm) or with the solvent reference relative to TMS employed as the internal standard (DMSO-d_6: 2.50 ppm; D_2O: 4.79 ppm). The following abbreviations are used to describe resonances: br, broad; s, singlet; d, doublet; dd, double doublet; q, quartet; m, multiplet; t, triplet. ^{13}C and DEPT spectra were recorded on Bruker Avance III (100 MHz and 126 MHz) spectrometers with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard. ^{31}P NMR NMR spectra were recorded on a Bruker Avance III (162 MHz) spectrometer with complete proton decoupling. Phosphorus chemical shifts are reported in ppm (δ) relative to an 85% H_3PO_4 external standard (H_3PO_4, δ 0.0 ppm). Melting points were determined using a Reichert-Jung Thermo Galen Kofler block or a Stanford Research Systems Optimelt MPA100 automated melting point system and are uncorrected. Microanalysis was carried out at the University of Bath microanalysis service. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre, Swansea, and at the University of Bath on VG Autospec or MicroTOF instruments. Flash column chromatography was performed on an ISCO CombiFlash Rf automated flash chromatography system using RediSep Rf disposable flash columns.

1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (4)

To a stirred suspension of *myo*-inositol (25.0 g, 139 mmol) in MeOH (250 mL) was added trimethyl orthoformate (100 mL), butanedione (25 mL, 285 mmol) and (±)-10-camphorsulphonic acid (0.5 g). The mixture was heated under N_2 at reflux for 96 h and the resulting cherry-red suspension was allowed to cool before triethylamine (1 mL) was added. The precipitate was filtered off, washed with MeOH (200 mL) and allowed to dry, giving crude diol **4** as a white solid (22.4 g, 85% pure by ^{1}H NMR). This material was crystallised from boiling $CHCl_3$/MeOH (1:1 v/v, 650 mL) and dried under vacuum at 60 °C to give **4** as colourless crystals (15.4 g, 37.7 mmol, 27%); R_f 0.24 (EtOAc); R_f 0.29 (2:1 $CHCl_3$–acetone); mp > 300°C with sublimation and decomposition; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.17 (6 H, s, CH_3), 1.18 (6 H, s, CH_3), 3.13 (6 H, s, OCH_3), 3.15 (6 H, s, OCH_3), 3.25 (1 H, dt, J = 5.6, 9.3 Hz, H-5), 3.35 (2 H, dd, J = 10.2, 2.4 Hz, H-1 and H-3), 3.67 (2 H, dd, J = 9.8, 9.8 Hz, H-4 and H-6), 3.76 (1 H, dt, J = 4.6, 2.4 Hz, H-2), 4.99 (1 H, d, J = 4.8 Hz, OH-2), 5.05 (1 H, d, J = 5.6 Hz, OH-5); ^{13}C NMR (DMSO-d_6) δ 17.59 (Me), 17.60 (Me), 47.08 (OMe), 47.36 (OMe), 67.59 (C-2), 68.37 (C-1 and C-3), 69.16 (C-5), 69.31 (C-4 and C-6), 98.40 (BDA quaternary C), 98.96 (BDA quaternary C); HRMS (*m*/*z*) $[M + Na]^+$ calcd. for $C_{18}H_{32}O_{10}$ 431.1888; found 431.1880; Anal. Calcd for $C_{18}H_{32}O_{10}$; C 52.93, H, 7.90; found C 52.6, H, 7.92.

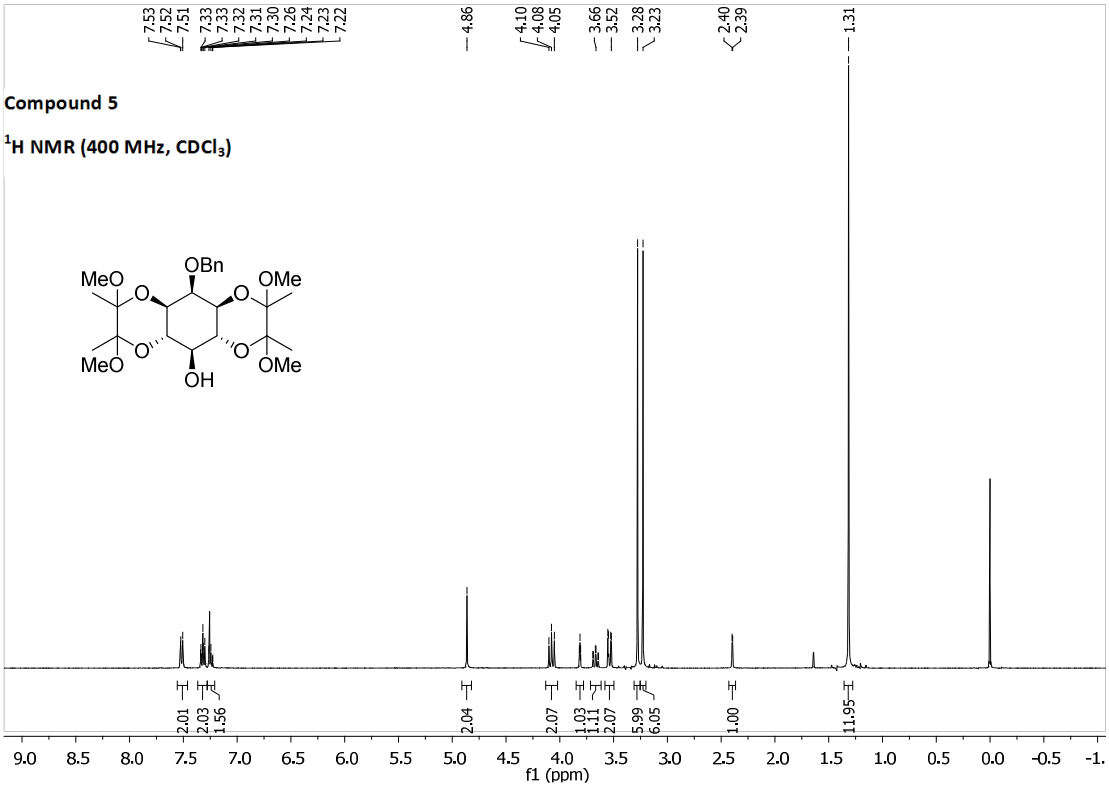


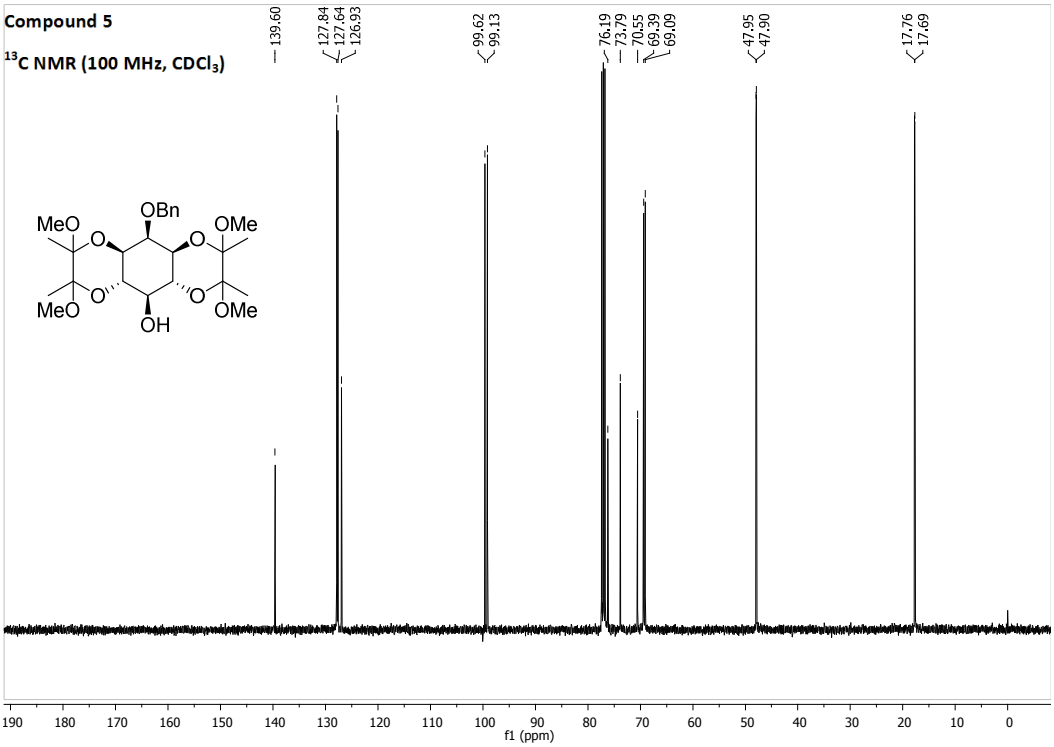


2-*O*-benzyl-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (5)

To a stirred suspension of diol (20.4 g, 50 mmol, dried *in vacuo* at 60 °C) in dry DMF (200 mL) was added sodium hydride (2.4 g of a 60% suspension in oil, 60 mmol). After 30 min, the mixture was cooled to 0 °C and benzyl bromide (6.5 mL, 55 mmol) was added dropwise over 30 min. The mixture was allowed to reach room temperature and stirring was continued for a further 18 h. Water (5 mL) was added and the mixture was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (300 mL) and washed with water (300 mL). The cloudy organic layer was dried over $MgSO_4$, filtered and concentrated to give an off-white solid (~23 g). The residue was taken up in CH_2Cl_2, adsorbed onto silica (70 g) and purified by flash chromatography (0 to 100% EtOAc in petroleum ether) to give pure 2-*O*-benzyl ether **5** as a white solid (13.6 g, 27.3 mmol, 55%). Crystals from boiling EtOH, m.p. 214–216 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (12 H, s, CH_3), 2.40 (1 H, d, *J* 2.0 Hz, OH-5), 3.23 (6 H, s, OCH_3), 3.28 (6 H, s, OCH_3), 3.54 (2 H, dd, *J* 10.2, 2.4 Hz, H-1 and H-3), 3.66 (1 H, td, *J* 9.4, 2.0, Hz, H-5), 3.81 (1 H, t, *J* 2.4 Hz, H-2), 4.08 (2 H, dd, *J* 10.0, 9.8 Hz, H-4 and H-6), 4.86 (2 H, s, O*C*H_2Ph), 7.22–7.26 (1 H, m, Ph), 7.29–7.34 (2 H, m, Ph), 7.50–7.53 (2 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 17.69 and 17.76 (CH_3), 47.90 and 47.95 (OCH_3), 69.09 (C-1 and C-3), 69.39 (C-4 and C-6), 70.55 (C-5), 73.79 (Ph*C*H_2O), 76.19 (C-2), 99.13 (BDA quaternary C), 99.62 (BDA

quaternary C), 126.93 (*para-C* of *Ph*CH_2O), 127.64 (*C*H of Ph), 127.84 (*C*H of Ph), 139.60 (*ipso-C* of *Ph*CH_2O); MS *m/z* (+ve ion FAB, relative intensity); 499.3 [(M + H)$^+$, 20%], 497.3 (30), 488.3 (80), 467.3 (50), 101.1 (60), 91.1 [$(C_7H_7)^+$, 100%]; HRMS (*m/z*) [M + Na]$^+$ calcd. for $C_{25}H_{38}O_{10}$; 521.2357; found 521.2337; Anal. Calcd for $C_{25}H_{38}O_{10}$; C 60.23, H, 7.68; found C 60.3, H, 7.44.





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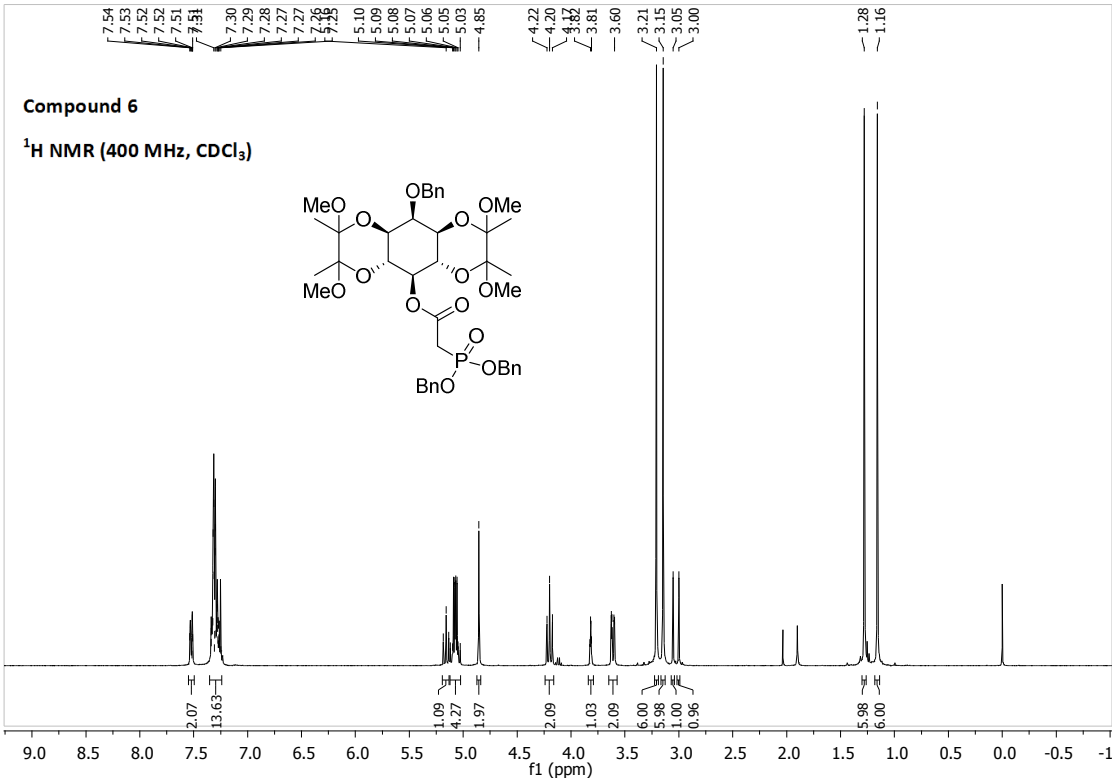
2-*O*-benzyl-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol 5-*O*-bis(benzyloxy)phosphorylacetate (6)

To a solution of 2-*O*-benzyl-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (**5)** (500 mg, 1.00 mmol) and dibenzylphosphonacetic acid[9] (352 mg, 1.10 mmol) in dry dichloromethane (10 mL) was added EDAC (230 mg, 1.20 mmol). The solution was stirred at room temperature under N_2 for 16 h. TLC (ethyl acetate/petrol 1:1) showed conversion of alcohol (R_f 0.32) into a less polar product (R_f 0.42). The solution was diluted with dichloromethane (50 mL), washed with 0.5 M HCl, saturated aqueous $NaHCO_3$ and brine (50 mL of each), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (ethyl acetate in petroleum ether, 0 to 100%) to give **6** as a colourless oil (757 mg, 0.945 mmol, 95%), which slowly crystallised; m.p. 135.5–137.5 °C (from chloroform/petroleum ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (6 H, s, CH_3), 1.28 (6 H, s, CH_3), 3.03 (2 H, d, $^2J_{HP}$ 21.6 Hz, CH_2P), 3.15 (6 H, s, OCH_3), 3.21 (6 H, s, OCH_3), 3.61 (2 H, dd, *J* 10.2, 2.4 Hz, H-1 and H-3), 3.82 (1 H, t, *J* 2.4 Hz, H-2), 4.20 (2 H, dd, *J* 10.1. 9.9 Hz, H-4 and H-6), 4.85 (2 H, s, OCH_2Ph), 5.16 (1 H, t, *J* 9.9 Hz, H-5), 5.03–5.12 (4 H, m, POCH_2Ph), 7.23–7.34 (13 H, m, Ph), 7.51–7.54 (2 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 17.62 and 17.66 (CH_3), 34.42 ($^1J_{CP}$ 136.8 Hz, CH_2P), 47.69 and 47.88 (OCH_3), 67.37 (C-4 and C-6), 68.06 ($^2J_{CP}$ 6.0 Hz, Ph*C*H_2OP), 69.18 (C-1 and C-3), 72.43 (C-5), 74.04 (Ph*C*H_2O), 76.12 (C-2), 99.12 (BDA quaternary C), 99.65 (BDA quaternary C), 127.06 (*para-C* of *Ph*CH_2O), 127.78 (*C*H of Ph), 127.89 (*ortho-C* of *Ph*CH_2OP), 127.92 (*C*H of Ph), 128.41 (*meta-C* of *Ph*CH_2O), 128.56 (*meta-C* of *Ph*CH_2OP), 135.96 ($^3J_{CP}$ 6.5 Hz, *ipso-C* of *Ph*CH_2OP), 139.48 (*ipso-C* of *Ph*CH_2O), 164.35 ($^2J_{CP}$ 5.3 Hz, C=O); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 20.39; HRMS (*m*/*z*) [M + Na]$^+$ calcd. for $C_{41}H_{53}O_{14}P$; 823.3065; found 823.3031; analysis (calcd., found for $C_{41}H_{53}O_{14}P$): C (61.49, 61.5), H (6.67, 6.70).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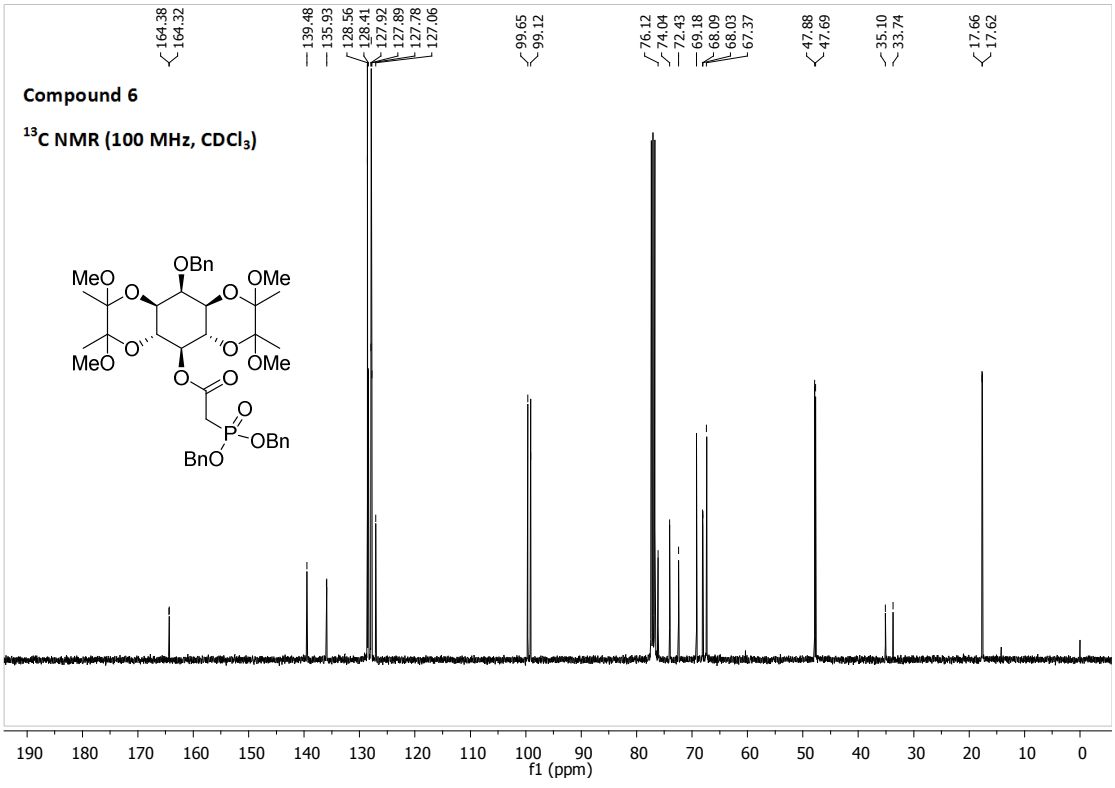
f1 (ppm)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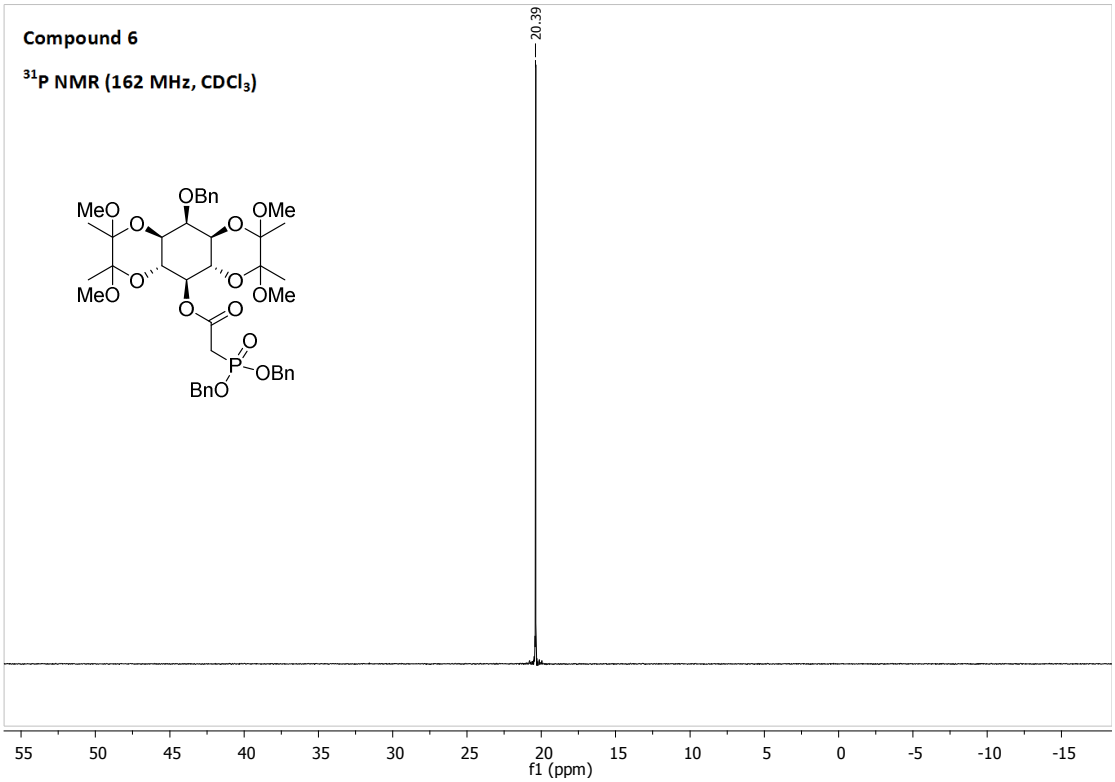
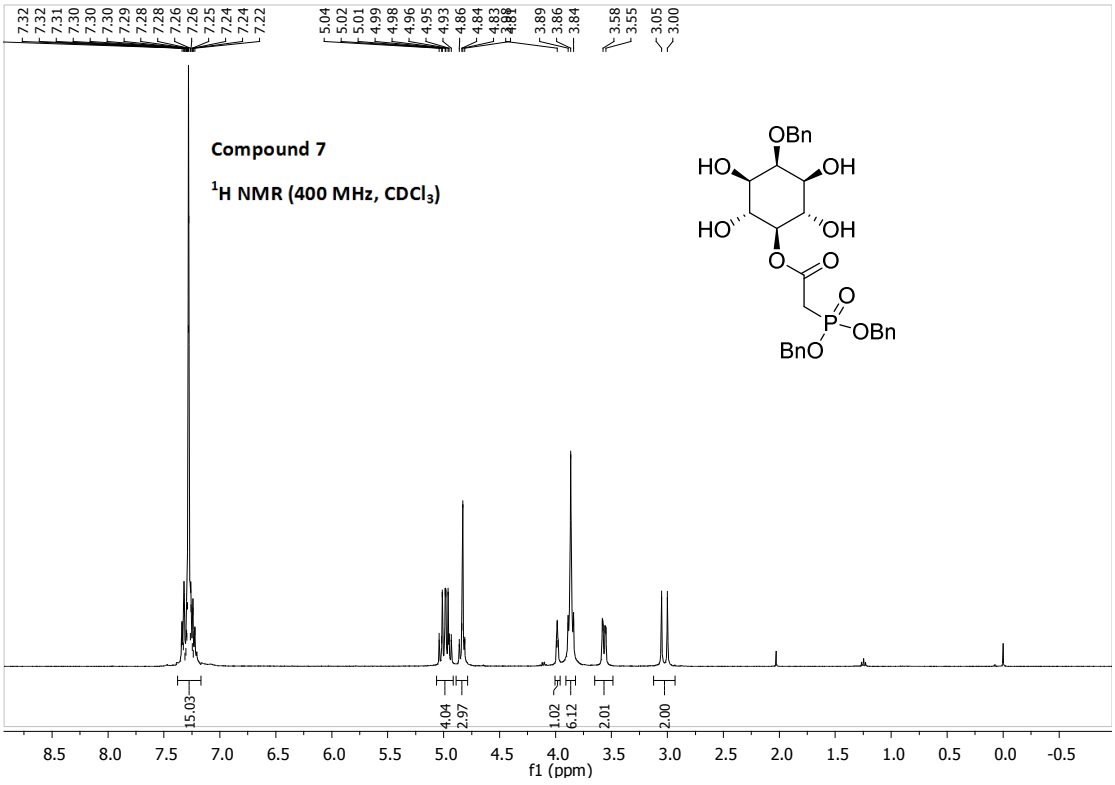
f1 (ppm)

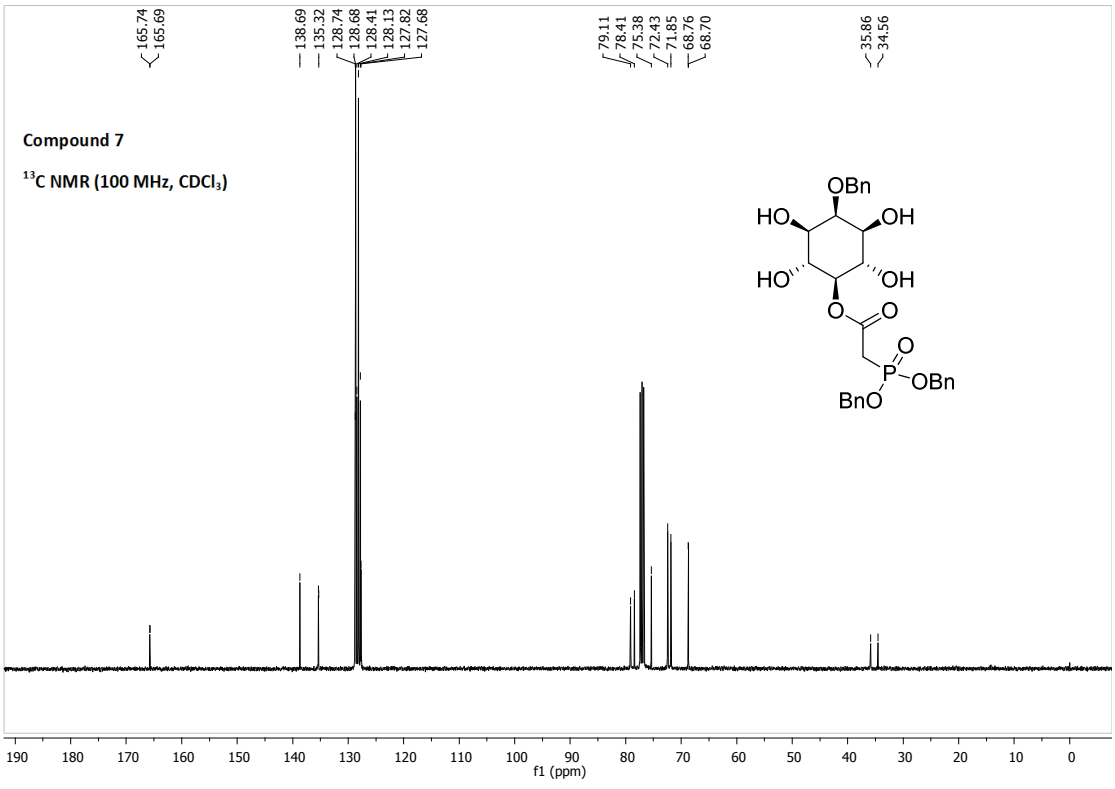


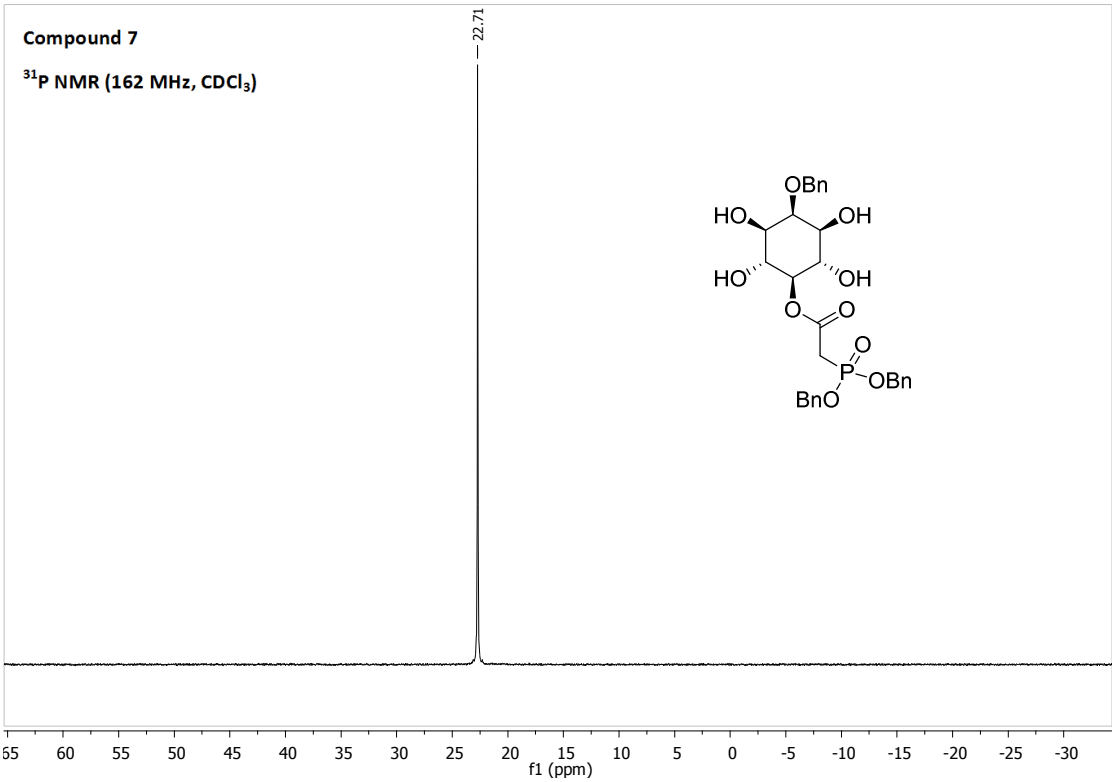
2-*O*-benzyl-*myo*-inositol 5-*O*-bis(benzyloxy)phosphorylacetate (7)

To a sample of **6** (244 mg, 0.305 mmol) was added 95% aqueous TFA (3 mL). The solution was stirred at room temperature for 5 min (yellow colour of butanedione will appear within seconds; it is important to keep reaction time short to minimise mono-debenzylation of the dibenzylphosphonate group) and the solvents were then rapidly removed by evaporation *in vacuo* (no heat) to give a solid residue. TLC (dichloromethane/methanol 10:1) showed conversion of starting material (R_f 0.70) into a major more polar product (R_f 0.22) together with traces of other polar products. The solid was dissolved in methanol, adsorbed onto silica (1 g) and purified by flash chromatography, eluting with a gradient of methanol (0 to 20%) in dichloromethane to give the tetraol **7** as a colourless oil (108 mg, 0.189 mmol, 62%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.03 (2 H, d, $^2J_{HP}$ 21.5 Hz, CH_2P), 3.57 (2 H, dd, J 9.8, 2.7 Hz, H-1 and H-3), 3.83–3.88 (6 H, m, H-4, H-6 and 4 × OH), 3.98 (1 H, t, J 2.7 Hz, H-2), 4.83 (2 H, s, OCH_2Ph), 4.84 (1 H, t, J 9.5 Hz, H-5), 4.93–5.04 (4 H, m, POCH_2Ph), 7.22–7.34 (15 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 35.21 ($^1J_{CP}$ 131.4 Hz, CH_2P), 68.73 ($^2J_{CP}$ 6.5 Hz, PhCH_2OP), 71.85 (C-4 and C-6), 72.43 (C-1 and C-3), 75.38 (PhCH_2O), 78.41 (C-5), 79.11 (C-2), 127.68 (*para-C* of *Ph*CH_2O), 127.82 (*para-C* of *Ph*CH_2OP), 128.13 (*ortho-C* of *Ph*CH_2OP), 128.41 (*ortho-C* of *Ph*CH_2O), 128.68 (*meta-C* of *Ph*CH_2O), 128.74 (*meta-C* of *Ph*CH_2OP), 135.35 ($^3J_{CP}$ 6.1 Hz, *ipso-C* of *Ph*CH_2OP),

138.69 (*ipso-C* of *Ph*CH_2O), 165.72 ($^2J_{CP}$ 5.8 Hz, C=O); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 22.71; HRMS (*m/z*) $[M + Na]^+$ calcd. for $C_{29}H_{33}O_{10}P$; 595.1704; found 595.1682.

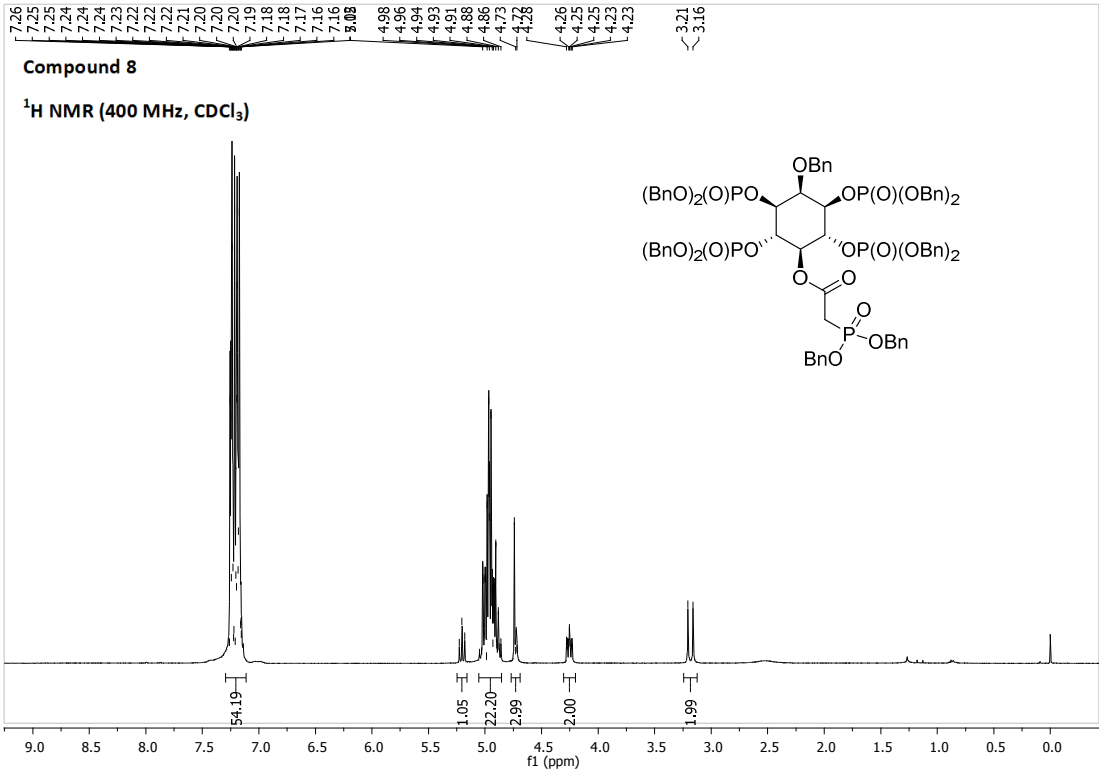


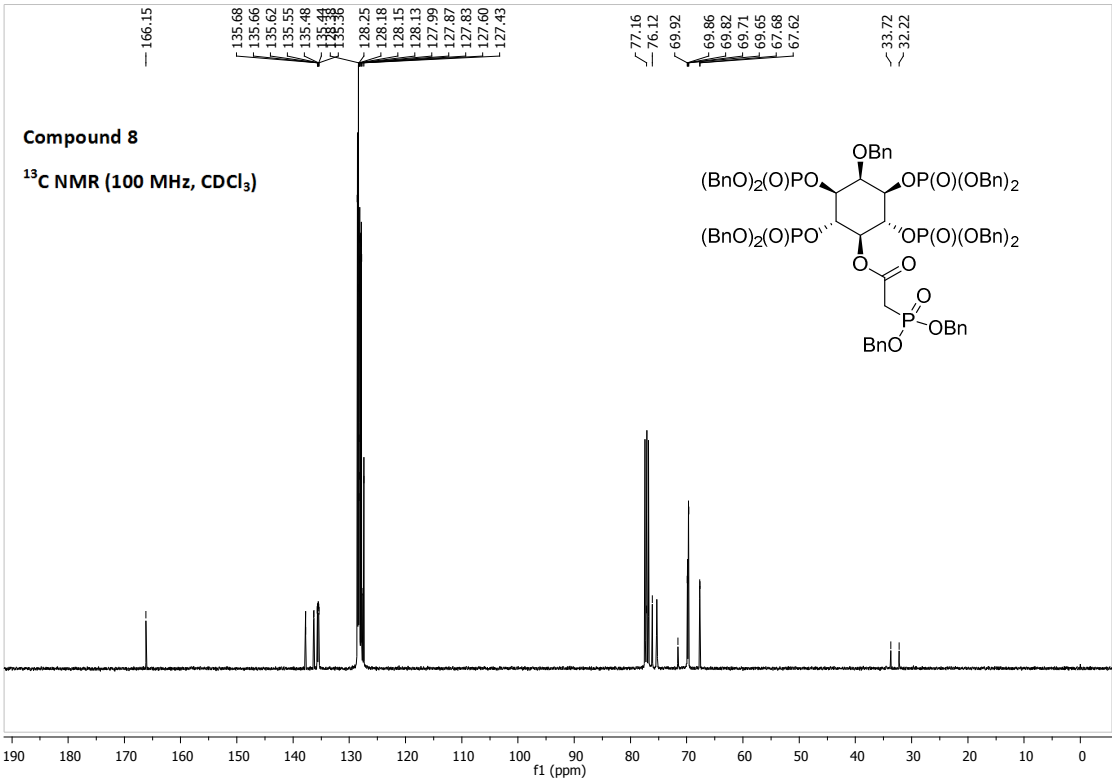


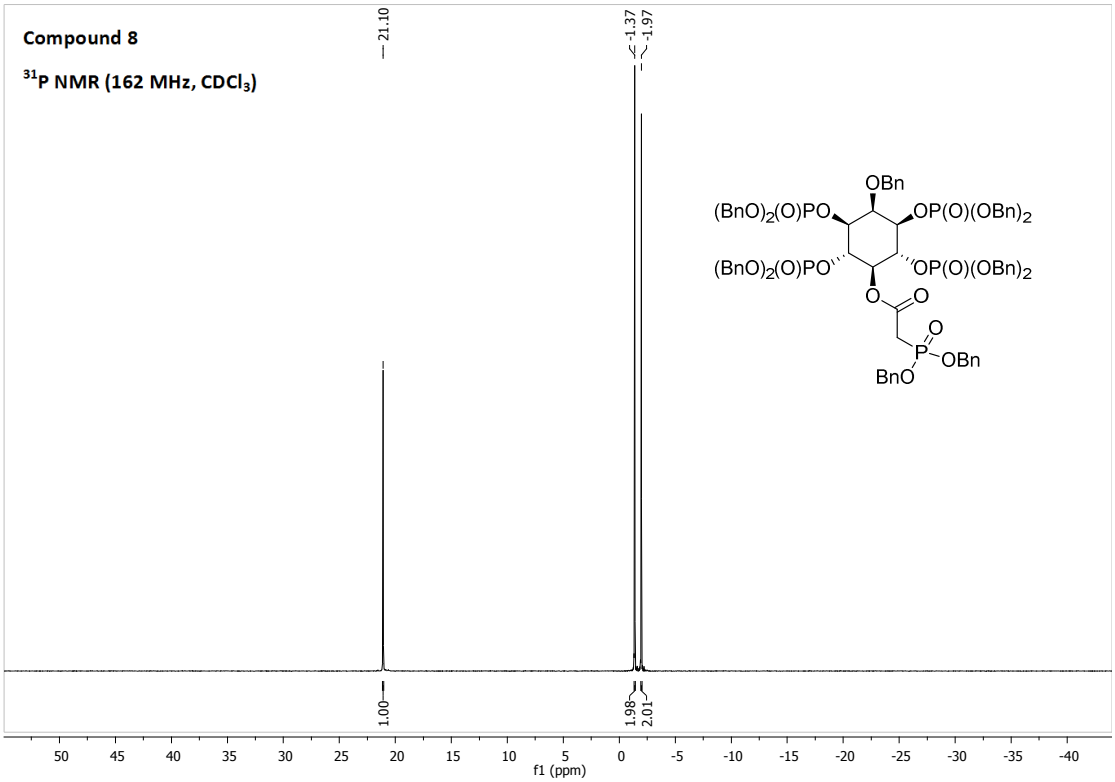

Compound 7
31P NMR (162 MHz, CDCl3)
22.71
OBn
HO
OH
HO
OH
O
O
O
P
OBn
BnO
65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30
f1 (ppm)

2-*O*-benzyl-*myo*-inositol 1,3,4,6-tetrakis(dibenzylphosphate) 5-bis(benzyloxy)phosphorylacetate (8)

To a solution of tetraol **7** (90 mg 0.157 mmol) in dry dichloromethane (3 mL) under N_2 was added 5-phenyl-1*H*-tetrazole (138 mg 0.942 mmol) and bis(benzyloxy)diisopropylaminophosphine (0.3 mL, 0.892 mmol). The mixture was stirred at room temperature for 1 h and then cooled to –78 ºC, before mCPBA (57%, 380 mg, 1.25 mmol) was added. The mixture was allowed to warm to room temperature and then diluted with EtOAc (30 mL). The clear solution was washed with 10% aq. Na_2SO_3 solution (2 × 30 mL), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (EtOAc in petroleum ether 0 to 100%) to give **8** as a colourless oil (240 mg, 0.149 mmole, 95%); TLC (EtOAc): R_f = 0.16; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.19 (2 H, d, $^2J_{HP}$ 18.0 Hz, CH_2P), 4.25 (2 H, ddd, *J* 10.0, 7.8 and 2.4 Hz, H-1 and H-3), 4.73 (1 H, t, *J* 2.4 Hz, H-2), 4.74 (2 H, s, OCH_2Ph), 4.86–5.05 (22 H, m, H-4, H-6 and POCH_2Ph), 5.20 (1 H, t, *J* 9.5 Hz, H-5), 7.14–7.27 (55 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 32.97 ($^1J_{CP}$ 151.2 Hz, CH_2P), 67.65 ($^2J_{CP}$ 6.5 Hz, Ph*C*H_2OPCH_2), 69.65–69.92 (with $^3J_{CP}$ couplings, Ph*C*H_2OPO), 71.54 (C-5), 75.31 (broad, C1, C-3, C-4 and C-6), 76.12 (Ph*C*H_2O), 77.16 (C-2), 127.43–128.57 (*C*H of Ph), 135.36–135.73 (overlapping signals with $^3J_{CP}$ couplings, *ipso-C* of *Ph*CH_2OP), 136.33 (*ipso-C* of *Ph*CH_2O), 166.15 (C=O); ^{31}P NMR ($CDCl_3$, 162 MHz) δ –1.97 [2 P, $(BnO)_2P(O)O$], –1.37, [2 P, $(BnO)_2P(O)O$], 21.10 [1 P, $(BnO)_2P(O)CH_2$]; HRMS (*m*/*z*) $[M + Na]^+$ calcd. for $C_{85}H_{85}O_{22}P_5$; 1635.4113; found 1635.4103.


Compound 8
^{1}H NMR (400 MHz, CDCl$_3$)
OBn
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
O
O
O
P
OBn
BnO
54.19
1.05
22.20
2.99
2.00
1.99
f1 (ppm)

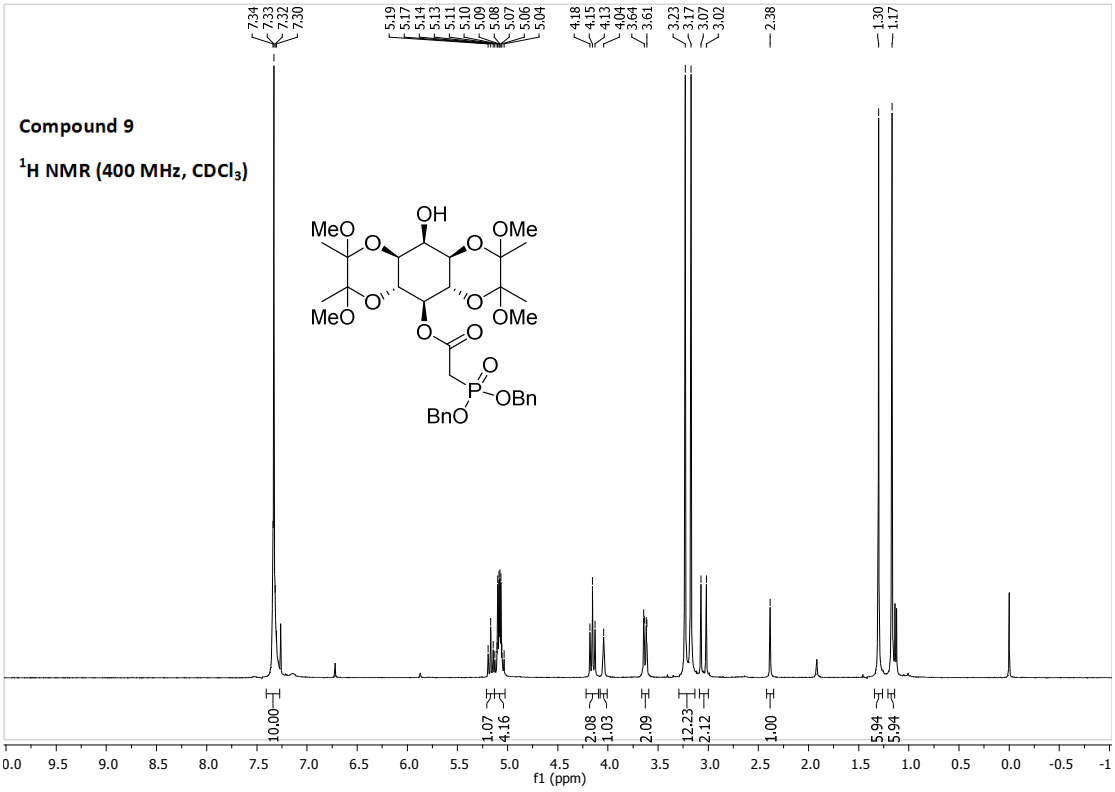

Compound 8
^{13}C NMR (100 MHz, CDCl$_3$)
OBn
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
O
O
O
P
OBn
BnO
f1 (ppm)

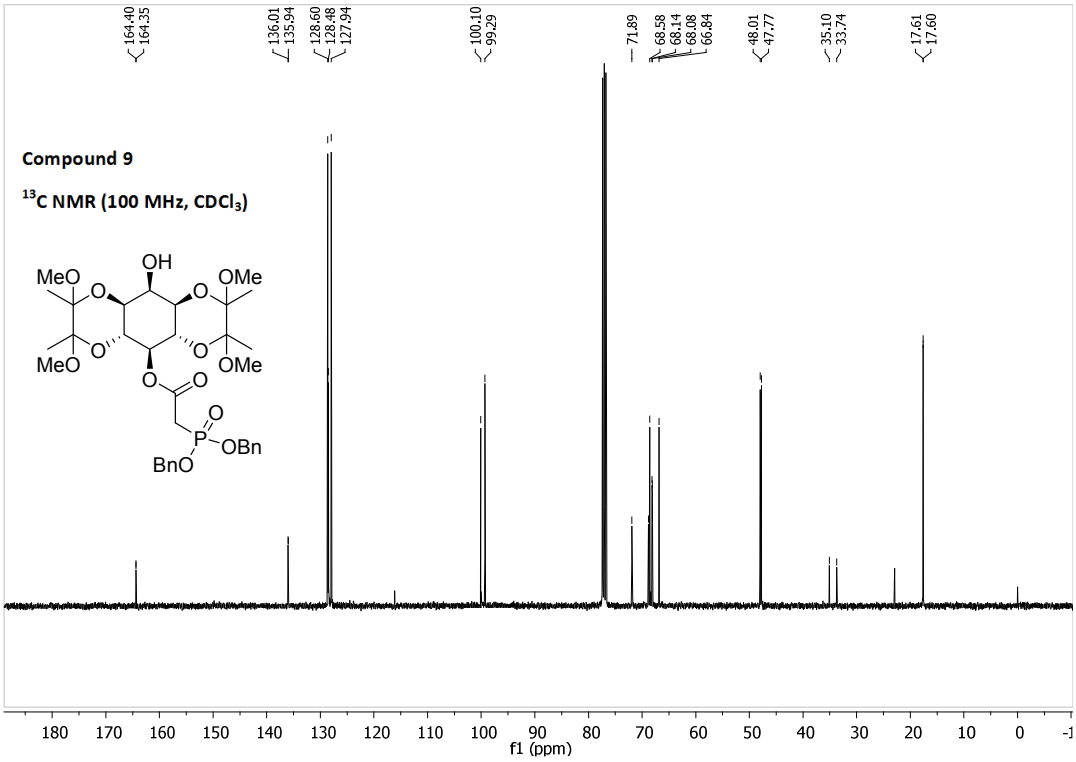


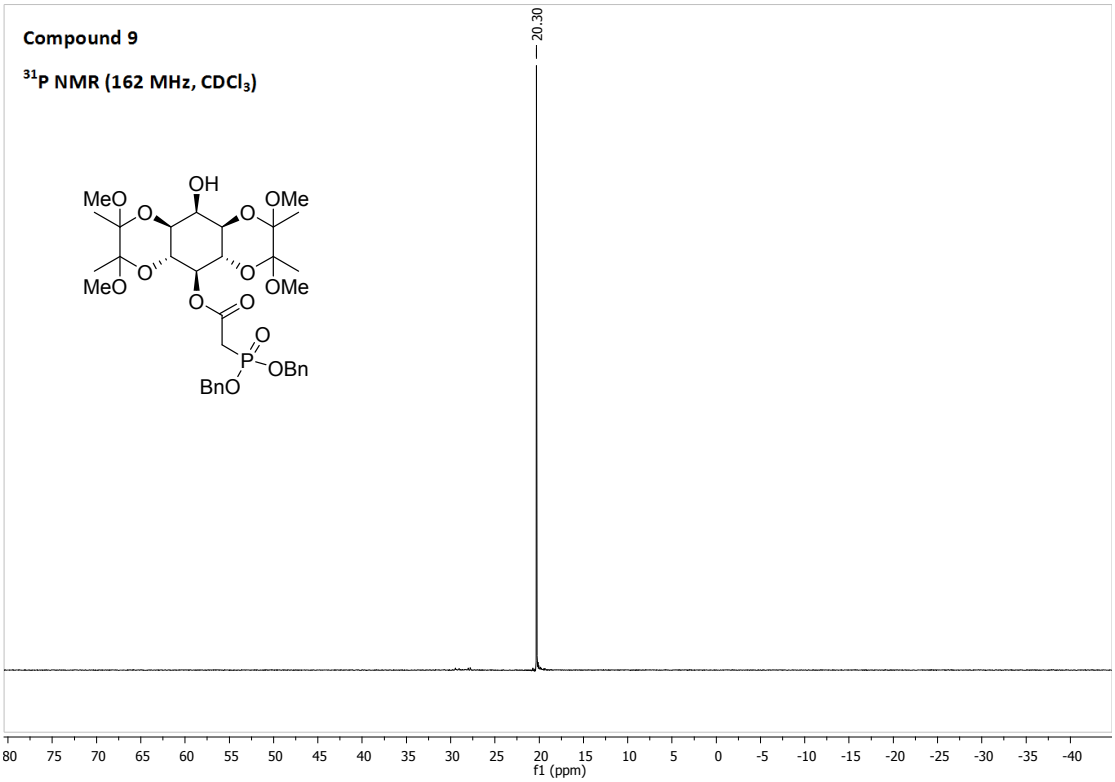
1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol 5-*O*-bis(benzyloxy)phosphorylacetate (9)

To a stirred suspension of finely ground **4** in dry dichloromethane (10 mL) was added a catalytic amount of DMAP (15 mg) and dibenzylphosphonoacetic acid[9] (380 mg, 1.18 mmol) followed by DCC (248 mg, 1.20 mmol). TLC (dichloromethane/ethyl acetate 1:1) showed conversion of diol (R_f 0.18) into a UV-active product (R_f 0.30) with traces of less polar products. After 2 h, the suspension was filtered through a plug of Celite to remove DCU and unreacted **4**. The filtrate was concentrated to give a white residue, which was purified by flash chromatography (ethyl acetate in dichloromethane, 0 to 100%) to give the product **9** as a white solid (521 mg, 0.733 mmol, 73% slightly contaminated with **4**. A small portion of **9** was recrystallised from diisopropyl ether/chloroform, m.p. 194–196 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (6 H, s, CH_3), 1.30 (6 H, s, CH_3), 2.38 (1 H, broad d, OH-2), 3.05 (2 H, d, $^2J_{HP}$ 21.6 Hz, CH_2P), 3.17 (6 H, s, OCH_3), 3.23 (6 H, s, OCH_3), 3.63 (2 H, dd, J 10.2, 2.6 Hz, H-1 and H-3), 4.04 (1 H, broad s, H-2), 4.15 (2 H, dd, J 10.1. 9.9 Hz, H-4 and H-6), 5.04–5.13 (4 H, ABX, J_{AB} 11.8 Hz, $^3J_{HP}$ 8.6 and 8.0 Hz, POCH_2Ph), 5.17 (1 H, t, J 9.9 Hz, H-5), 7.30–7.34 (10 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 17.60 and 17.61 (CH_3), 34.42 ($^1J_{CP}$ 137.0 Hz, CH_2P), 47.77 and 48.01 (OCH_3), 66.84 (C-4 and C-6), 68.11 ($^2J_{CP}$ 6.1 Hz, PhCH_2OP), 68.58 (C-1 and C-3), 68.83 (C-2), 71.89 (C-5), 99.29

(BDA quaternary C), 100.10 (BDA quaternary C), 127.95 (*ortho-C* of *Ph*CH_2OP), 128.48 (*para-C* of *Ph*CH_2OP), 128.60 (*meta-C* of *PhC*H_2OP), 135.98 ($^3J_{CP}$ 6.5 Hz, *ipso-C* of *Ph*CH_2OP), 164.38 ($^2J_{CP}$ 5.1 Hz, C=O); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 20.30; HRMS (*m*/*z*) $[M + Na]^+$ calcd. for $C_{34}H_{47}O_{14}P$; 733.2596; found 733.2579; analysis (calcd., found for $C_{34}H_{47}O_{14}P$): C (57.46, 57.4), H (6.67, 6.63).



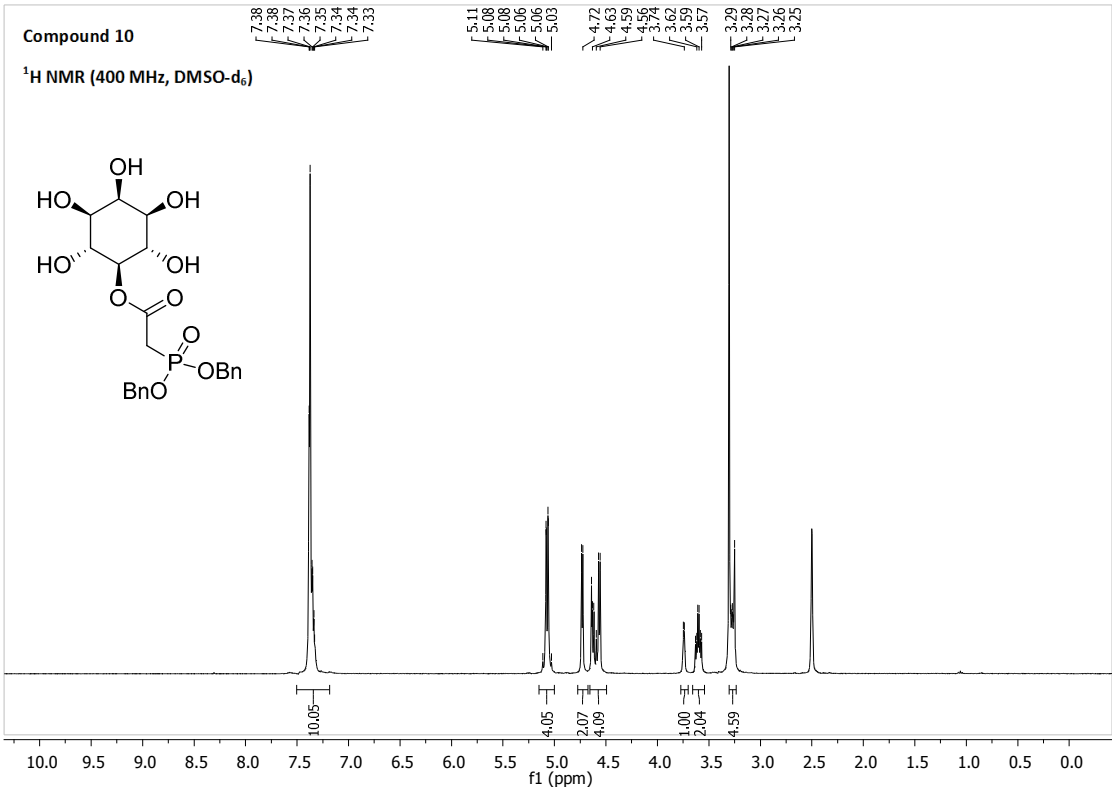


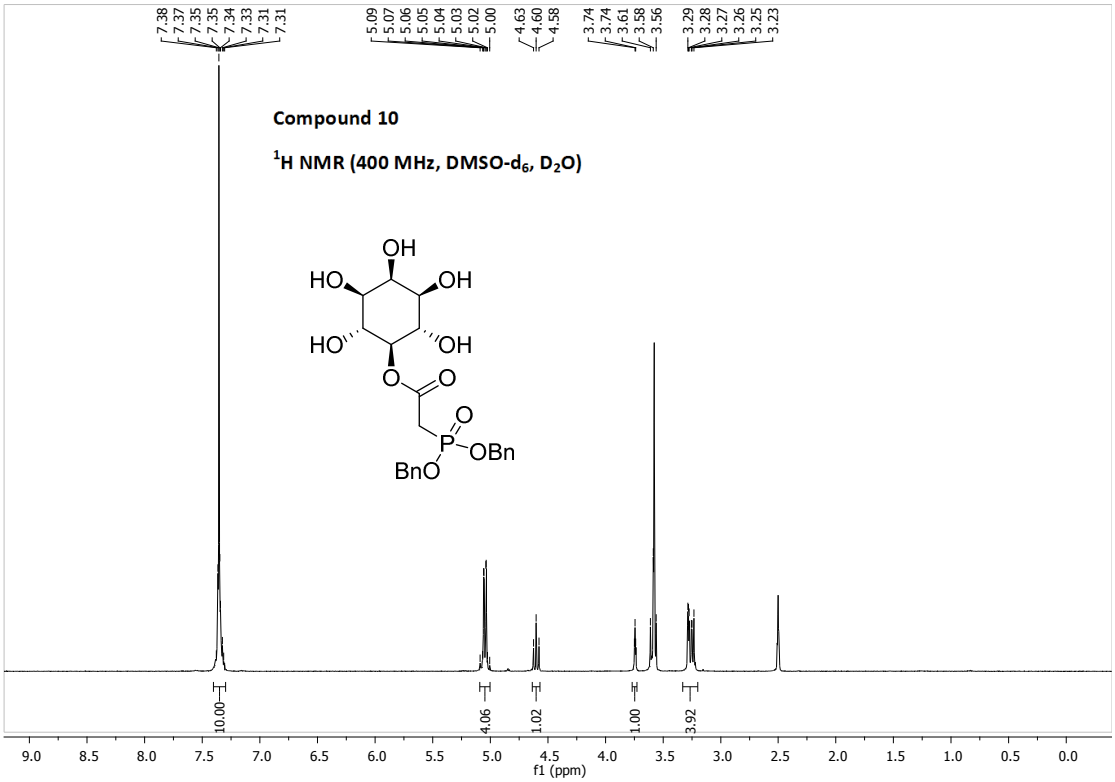


***myo*-inositol 5-*O*-bis(benzyloxy)phosphorylacetate (10)**

To a sample of **9** (350 mg, 0.492 mmol) was added 95% aqueous TFA (5 mL). The solution was stirred at room temperature for 5 min (yellow colour of butanedione will appear within seconds; it is important to keep reaction time short to minimise mono-debenzylation of the dibenzylphosphonate group) and the solvents were then rapidly removed by evaporation *in vacuo* (no heat) to give a solid residue. TLC (dichloromethane/methanol 8:1) showed conversion of starting material (R_f 0.80) into a more polar product (R_f 0.20) together with traces of other polar products. The solid was dissolved in methanol, adsorbed onto silica (1 g) and purified by flash chromatography, eluting with a gradient of methanol (0 to 20%) in dichloromethane to give pentaol **10** as a colourless oil, which slowly crystallised (119 mg, 0.247 mmol, 50%, m.p. 152.5–154.5 °C (from ethanol); ^{1}H NMR [$(CD_3)_2SO$, 400 MHz] δ 3.25–3.29 (4 H, m, buried, CH_2P, H-1 and H-3), 3.60 (ddd, J 9.6, 9.6, 4.8 Hz, H-4 and H-6), 3.72–3.77 (1 H, m, H-2), 4.56 (2 H, d, J 5.8 Hz, OH-1 and OH-3), 4.60 (1 H, t, J 9.6 Hz, H-5), 4.64 (1 H, d, J 3.5 Hz, OH-2), 4.73 (2 H, d, J 4.8 Hz, OH-4 and OH-6), 5.03–5.11 (4 H, m, POCH_2Ph), 7.31–7.38 (10 H, m, Ph); ^{1}H NMR [$(CD_3)_2SO/D_2O$, 400 MHz] δ 3.26 (2 H, d, $^2J_{HP}$ 20.8 Hz, CH_2P), 3.265 (2 H, dd, J 9.7, 2.7 Hz, H-1 and H-3), 3.58 (2 H, dd, J 9.7. 9.5 Hz, H-4 and H-6), 3.74 (t, J 2.7 Hz, H-2), 4.60 (1 H, t, J 9.6 Hz, H-5), 5.00–5.09 (4 H, m, POCH_2Ph), 7.32–7.38 (10 H, m, Ph); ^{13}C NMR

[$(CD_3)_2SO/D_2O$, 100 MHz] δ 34.05 ($^1J_{CP}$ 132.8 Hz, CH_2P), 67.71 ($^2J_{CP}$ 6.1 Hz, Ph*C*H_2OP), 70.56 (C-4 and C-6), 71.53 (C-1 and C-3), 72.54 (C-2), 78.92 (C-5), 128.09 (*ortho-C* of *Ph*CH_2OP), 128.58 (*para-C* of *Ph*CH_2OP), 128.75 (*meta-C* of *Ph*CH_2OP), 136.39 ($^3J_{CP}$ 6.6 Hz, *ipso-C* of *Ph*CH_2OP), 165.33 ($^2J_{CP}$ 5.1 Hz, C=O); ^{31}P NMR [$(CD_3)_2SO/D_2O$, 162 MHz] δ 22.25; HRMS (*m*/*z*) $[M + Na]^+$ calcd. for $C_{22}H_{27}O_{10}P$; 505.1234; found 505.1219; analysis (calcd., found for $C_{22}H_{27}O_{10}P$): C (54.77, 54.5), H (5.64, 5.62).




Compound 10
^{1}H NMR (400 MHz, DMSO-d$_6$, D$_2$O)
OH
HO
OH
HO
OH
O
O
O
P
OBn
BnO
7.38
7.37
7.35
7.35
7.34
7.33
7.31
7.31
5.09
5.07
5.06
5.05
5.04
5.03
5.02
5.00
4.63
4.60
4.58
3.74
3.74
3.61
3.58
3.56
3.29
3.28
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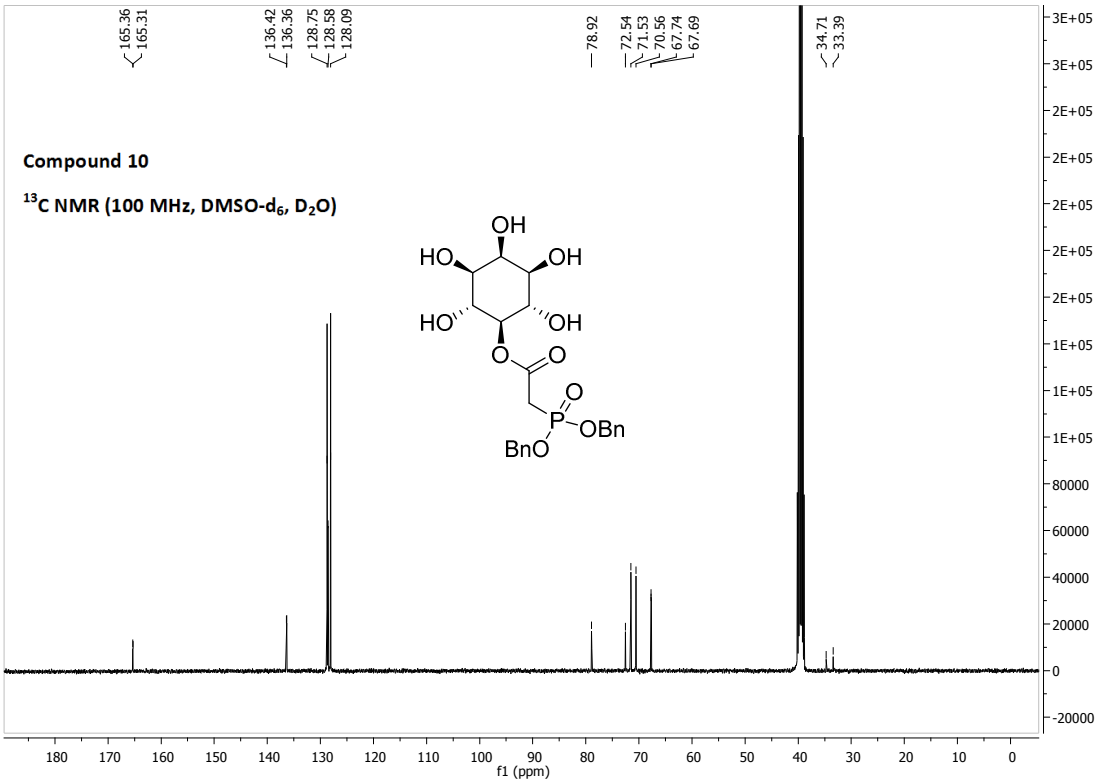
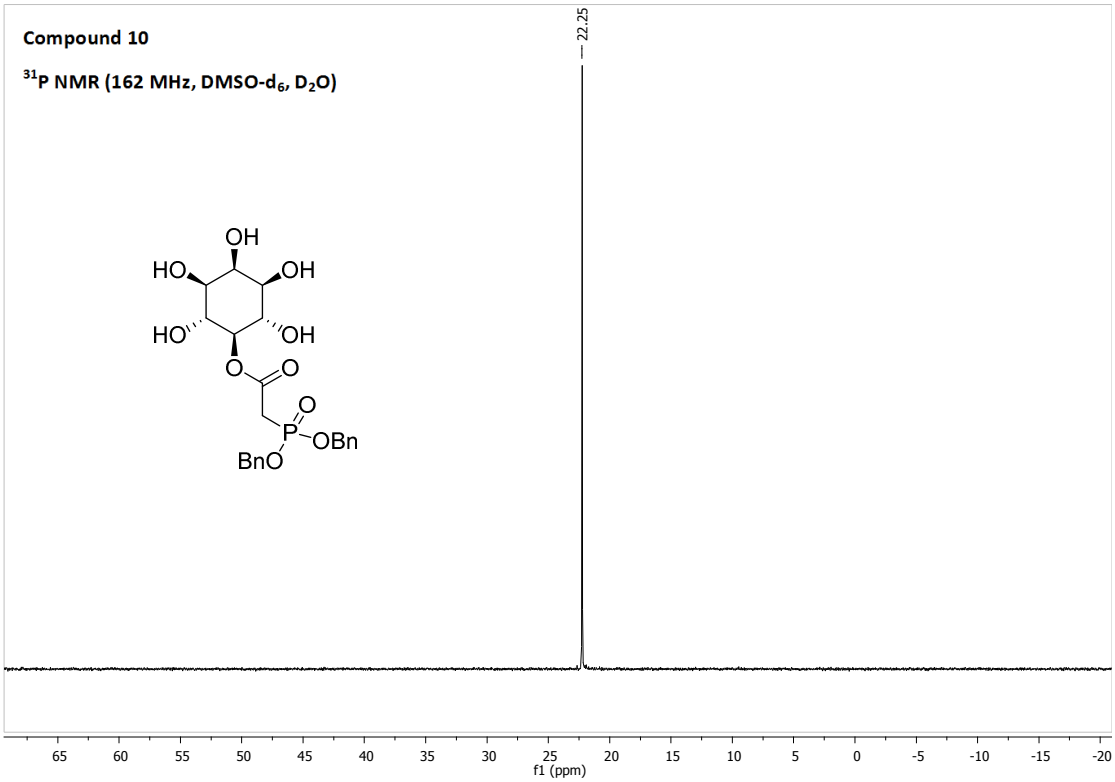
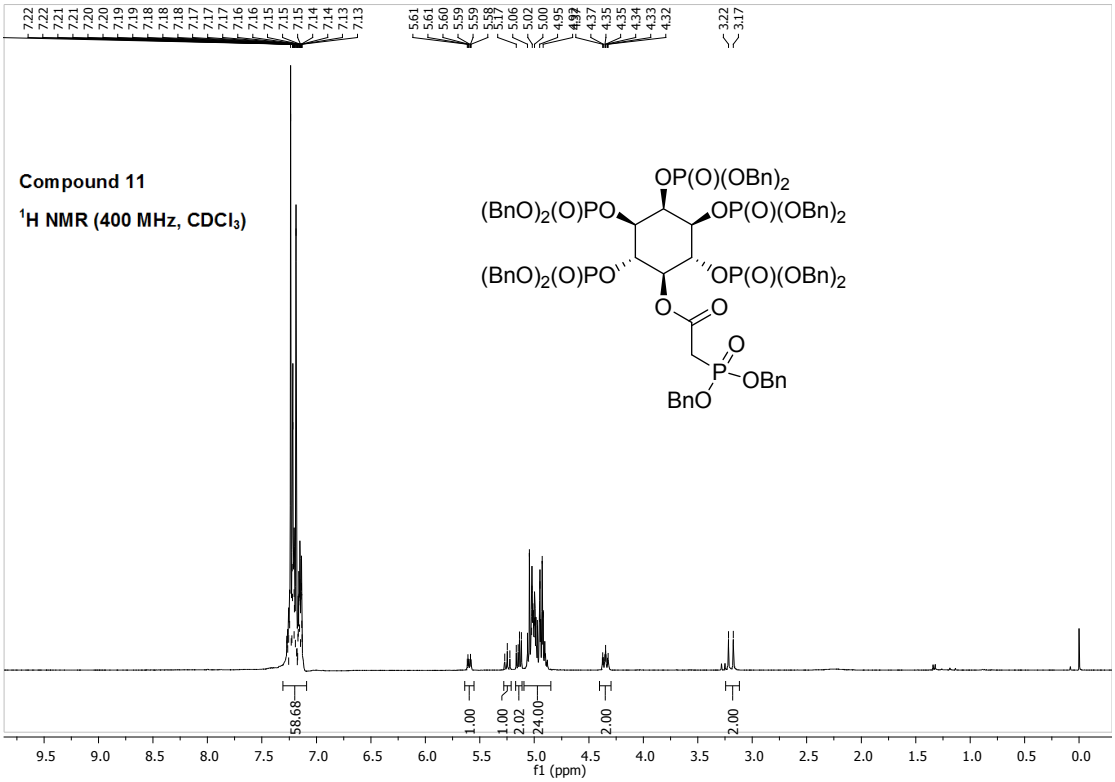
3.26
3.25
3.23
10.00
4.06
1.02
1.00
3.92
f1 (ppm)

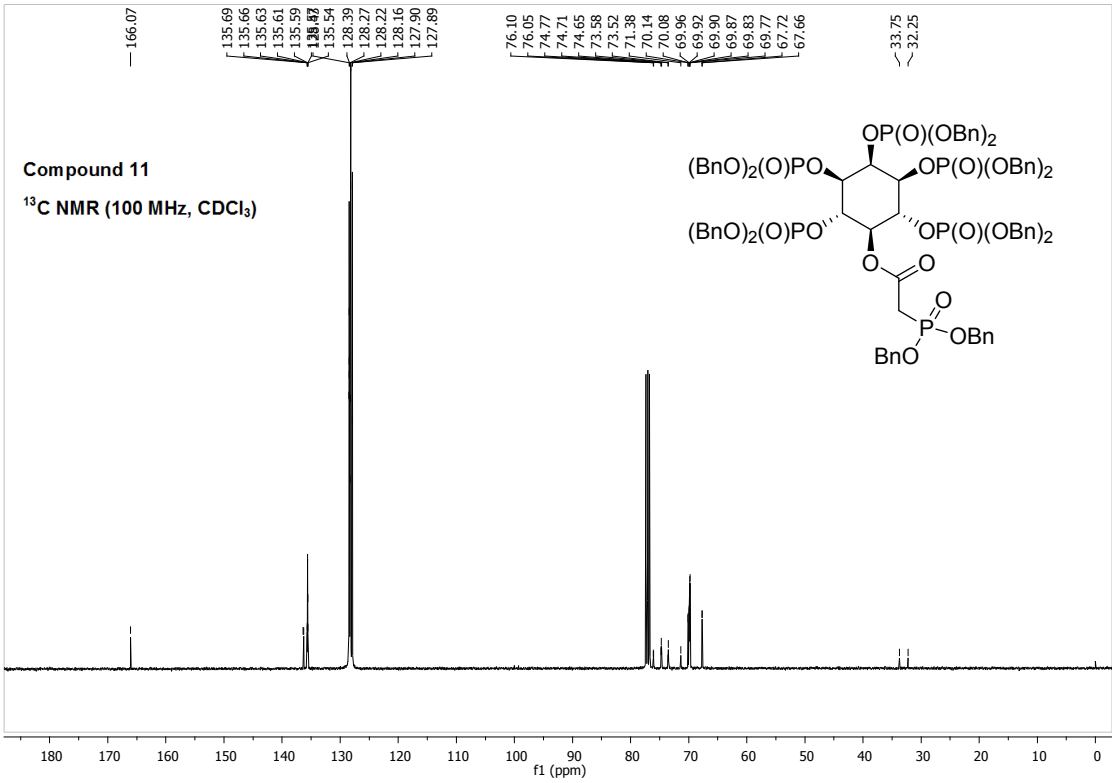

Compound 10
^{13}C NMR (100 MHz, DMSO-d$_6$, D$_2$O)
OH
HO
OH
HO
OH
O
O
O
P
OBn
BnO
165.36
165.31
136.42
136.36
128.75
128.58
128.09
78.92
72.54
71.53
70.56
67.74
67.69
34.71
33.39
f1 (ppm)

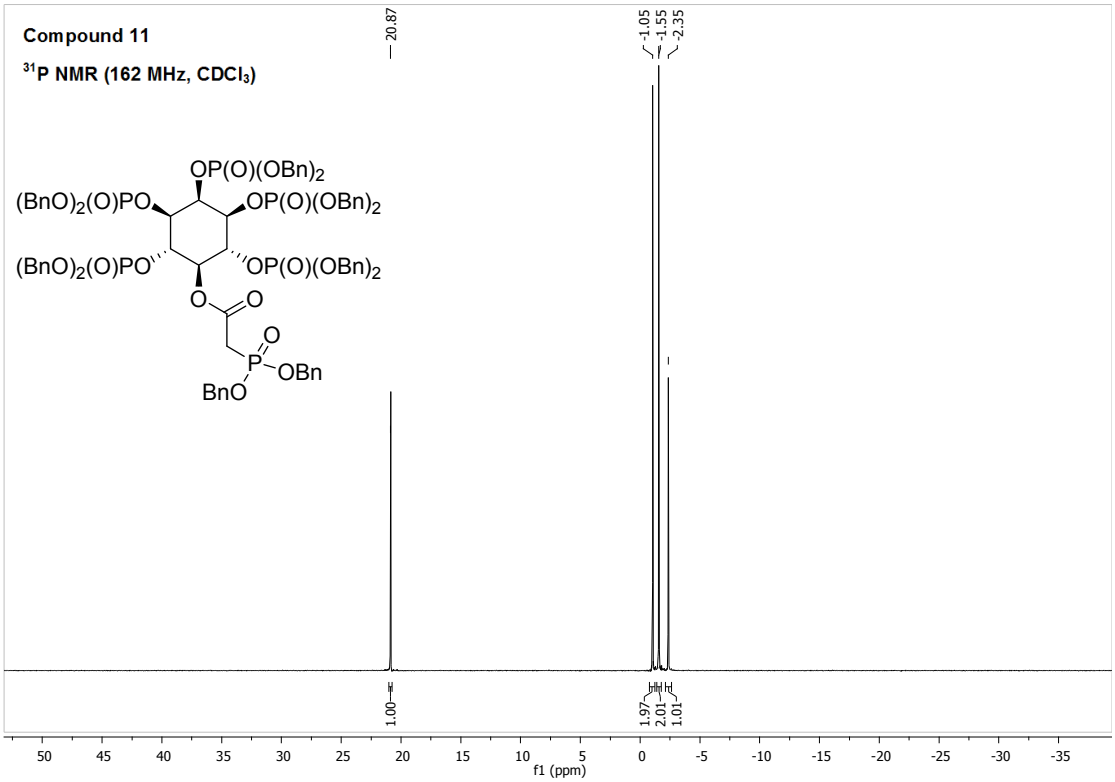

Compound 10
31P NMR (162 MHz, DMSO-d6, D2O)
22.25
OH
HO
OH
HO
OH
O
O
O
P
OBn
BnO
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
-10
-15
-20
f1 (ppm)

***myo*-inositol 1,2,3,4,6-pentakis(dibenzylphosphate) 5-bis(benzyloxy)phosphorylacetate (11)**

To a stirred suspension of pentaol **10** (65 mg 0.135 mmol) in dry dichloromethane (3 mL) under N_2 was added 5-phenyl-1*H*-tetrazole (146 mg 1.00 mmol) and bis(benzyloxy)diisopropylaminophosphine (0.3 mL, 0.9 mmol). The mixture was stirred at room temperature for 2 h and then cooled to –78 ºC, before MCPBA (57%, 400 mg, 1.32 mmol) was added. The mixture was allowed to warm to room temperature and then diluted with EtOAc (30 mL). The clear solution was washed with 10% aq. Na_2SO_3 solution (2 × 30 mL), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (EtOAc in petroleum ether 0 to 100%) to give **11** as a colourless oil which slowly crystallised (202 mg, 0.113 mmole, 84%); m.p. 100–102 °C (from diethyl ether/petroleum ether); TLC (EtOAc): R_f = 0.14; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.19 (2 H, d, $^2J_{HP}$ 18.0 Hz, CH_2P), 4.31–4.38 (2 H, m, H-1 and H-3), 4.87–5.08 (24 H, m, H-4, H-6 and POCH_2Ph), 5.12–5.17 (2 H, m, POCH_2Ph), 5.25 (1 H, t, *J* 9.6 Hz, H-5), 5.60 (1 H, dt, *J* 9.1, 2.3 Hz, H-2), 7.12–7.27 (55 H, m, Ph); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 33.00 ($^1J_{CP}$ 151.2 Hz, CH_2P), 67.68 ($^2J_{CP}$ 6.0 Hz, Ph*C*H_2OPCH_2), 69.75–70.15 (with $^3J_{CP}$ couplings, Ph*C*H_2OPO), 71.38 (C-5), 73.51 (C-1 and C-3), 74.71 (C-4 and C-6), 76.07 (C-2), 127.89–128.50 (*C*H of Ph), 135.54–135.69 (overlapping signals with $^3J_{CP}$ couplings, *ipso-C* of *Ph*CH_2OP), 136.33 ($^3J_{CP}$ 6.4 Hz, *ipso-C* of *Ph*CH_2OP), 166.07 (C=O); ^{31}P NMR ($CDCl_3$, 162 MHz) δ –2.35 [1 P, $(BnO)_2P$(O)O], –1.55 [2 P, $(BnO)_2P$(O)O], –1.05 [2 P, $(BnO)_2P$(O)O], 20.87 [1 P, $(BnO)_2P$(O)CH_2]; analysis (calcd., found for $C_{92}H_{92}O_{25}P_6$): C (61.95, 62.0), H (5.20, 5.17).


Compound 11
^{1}H NMR (400 MHz, CDCl$_3$)
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
O
O
O
P
OBn
BnO
f1 (ppm)

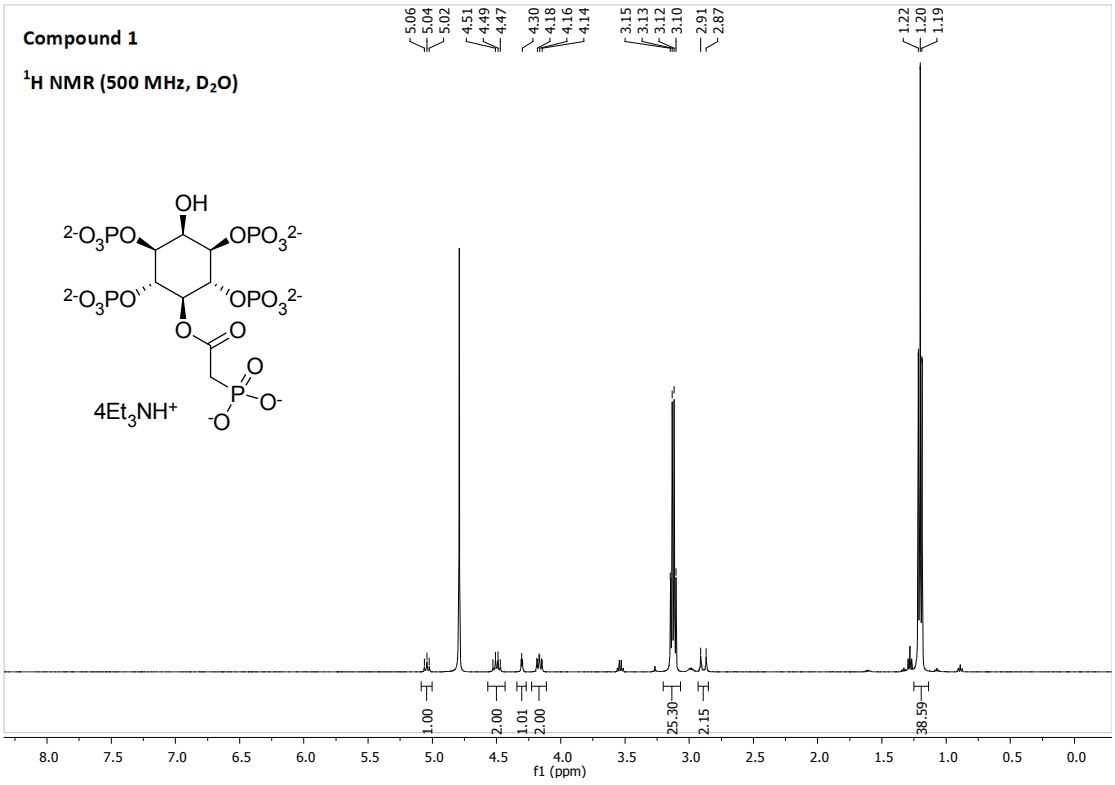

Compound 11
^{13}C NMR (100 MHz, CDCl$_3$)
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
(BnO)$_2$(O)PO
OP(O)(OBn)$_2$
O
O
O
P
OBn
BnO
f1 (ppm)

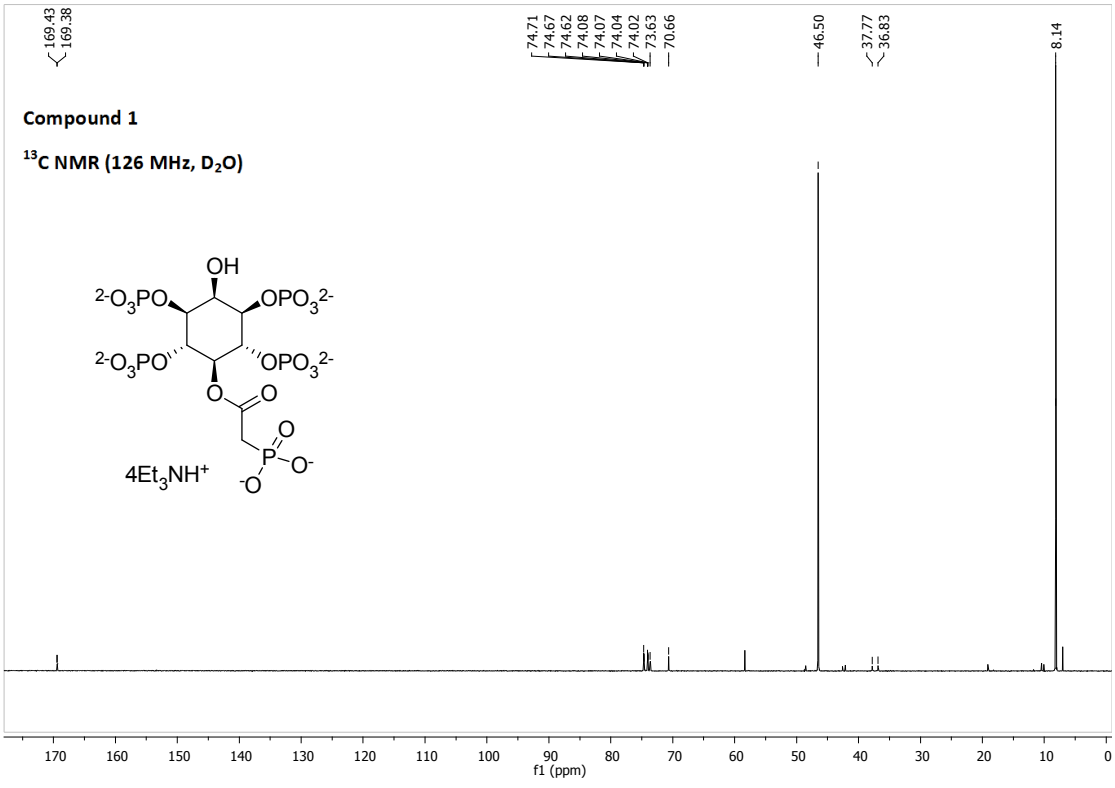


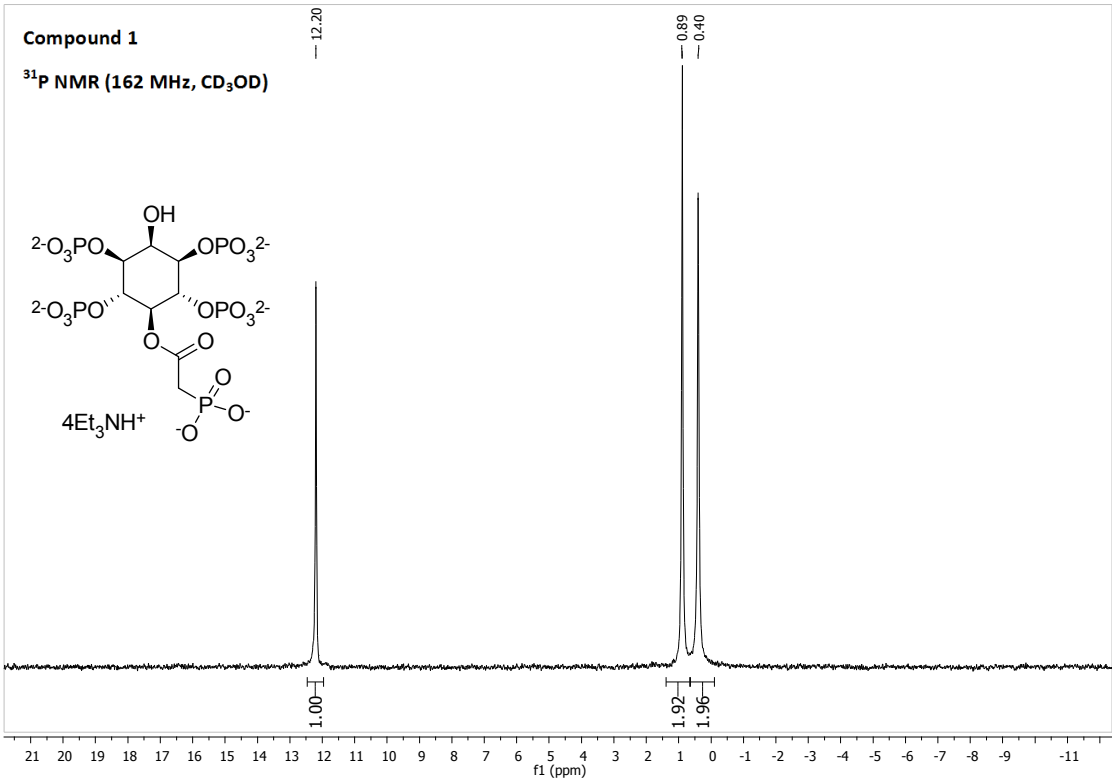
***myo*-inositol 1,3,4,6-tetrakisphosphate-5-phosphorylacetate (1)**

To a solution of **8** (85 mg, 52 μmol) in MeOH (8 mL) and deionised water (2 mL) was added palladium hydroxide on activated charcoal (Fluka, 20%, 50% water, 50 mg). The suspension was shaken in a Parr hydrogenator under H_2 (50 p.s.i.) for 18 h. The catalyst was removed by filtration through a PTFE filter, giving a colourless solution, which was concentrated under reduced pressure. A ^{1}H NMR spectrum in D_2O of the residue at this stage showed no residual aromatic signals, indicating that hydrogenolysis was complete. The residue was purified by ion-exchange chromatography on Q Sepharose Fast Flow resin eluting with a gradient of aqueous TEAB (0 to 2.0 moldm^{-3}) to give the triethylammonium salt of **1** (44 mg, 41 μmol, 79%); ^{1}H NMR (D_2O, 500 MHz, HDO ref at 4.79) δ 1.20 (~39 H, t, J 7.4 Hz, CH_3 of TEA^+), 2.89 (2 H, d, $^2J_{HP}$ 20.7 Hz, CH_2P), 3.13 (~25 H, q, J 7.4 Hz, CH_2 of TEA^+), 4.17 (2 H, ddd, J 9.8, 9.7, 2.6 Hz, H-1 and H-3), 4.30 (1 H, t, J 2.6 Hz, H-2), 4.50 (2 H, ddd, J 9.6, 9.5, 9.5 Hz, H-4 and H-6), 5.04 (1 H, t, J 9.6 Hz, H-5); ^{13}C NMR (D_2O, 126 MHz) δ 8.14 (CH_3 of TEA^+), 37.30 ($^1J_{CP}$ 118.1 Hz, CH_2P), 46.50 (CH_2 of TEA^+), 70.66 (C-2), 73.63 (C-5), 74.06 (J_{CP} 5.7, 2.0 Hz, C-1 and C-3), 74.67 (J_{CP} 5.7, 5.6 Hz, C-4 and C-6), 169.40 ($^2J_{CP}$ 6.4 Hz, C=O); ^{31}P NMR (CD_3OD, 162 MHz) δ 0.40 (2 P,

phosphate P), 0.89 (2 P, phosphate P), 12.20 (1 P, phosphonate P); HRMS (*m*/*z*) $[M]^-$ calcd. for $C_8H_{18}O_{22}P_5$; 620.8983; found 620.8988.



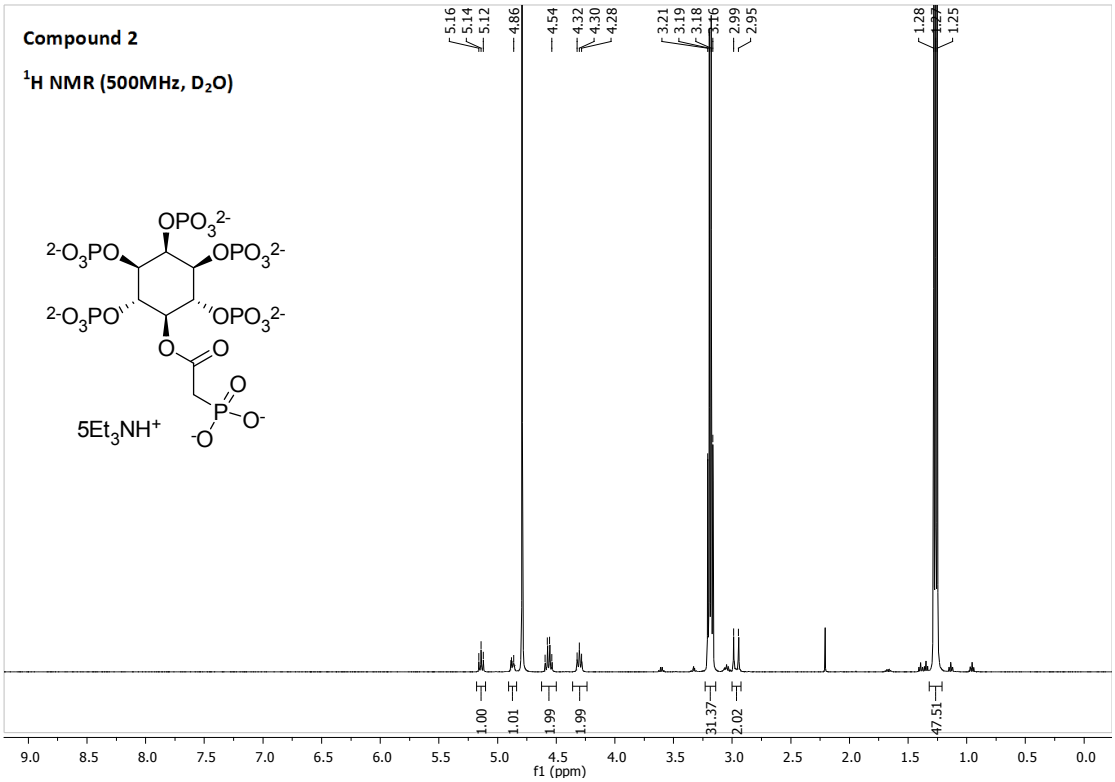


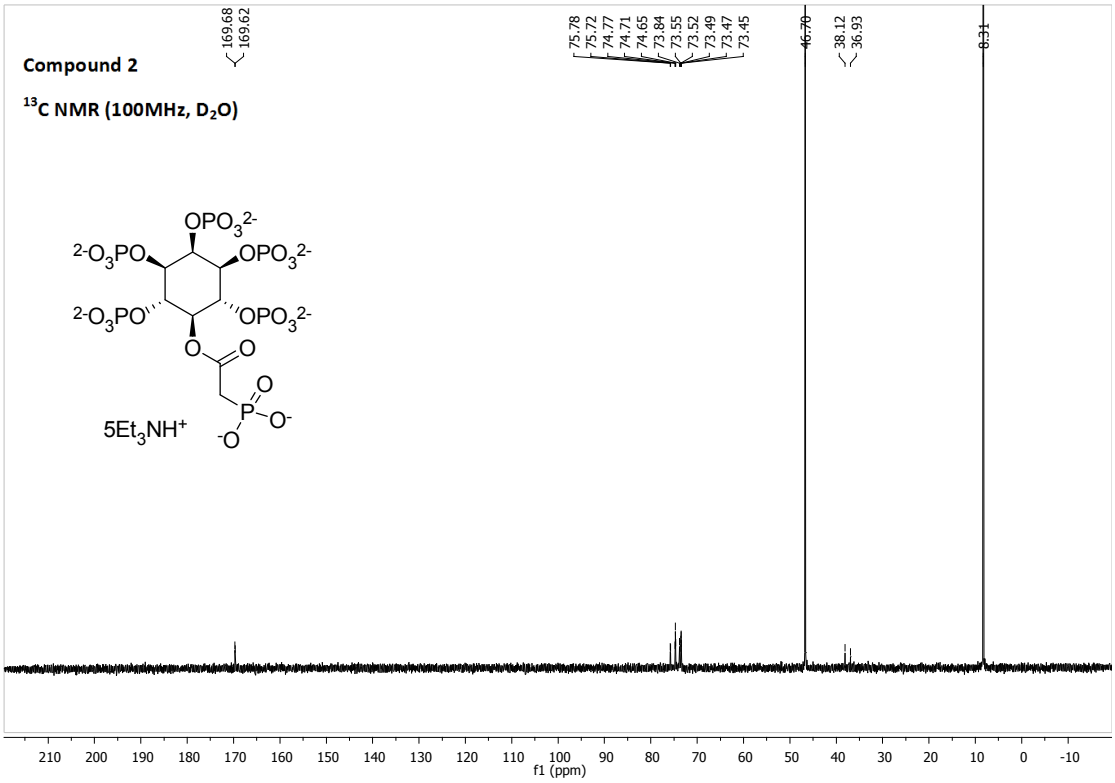


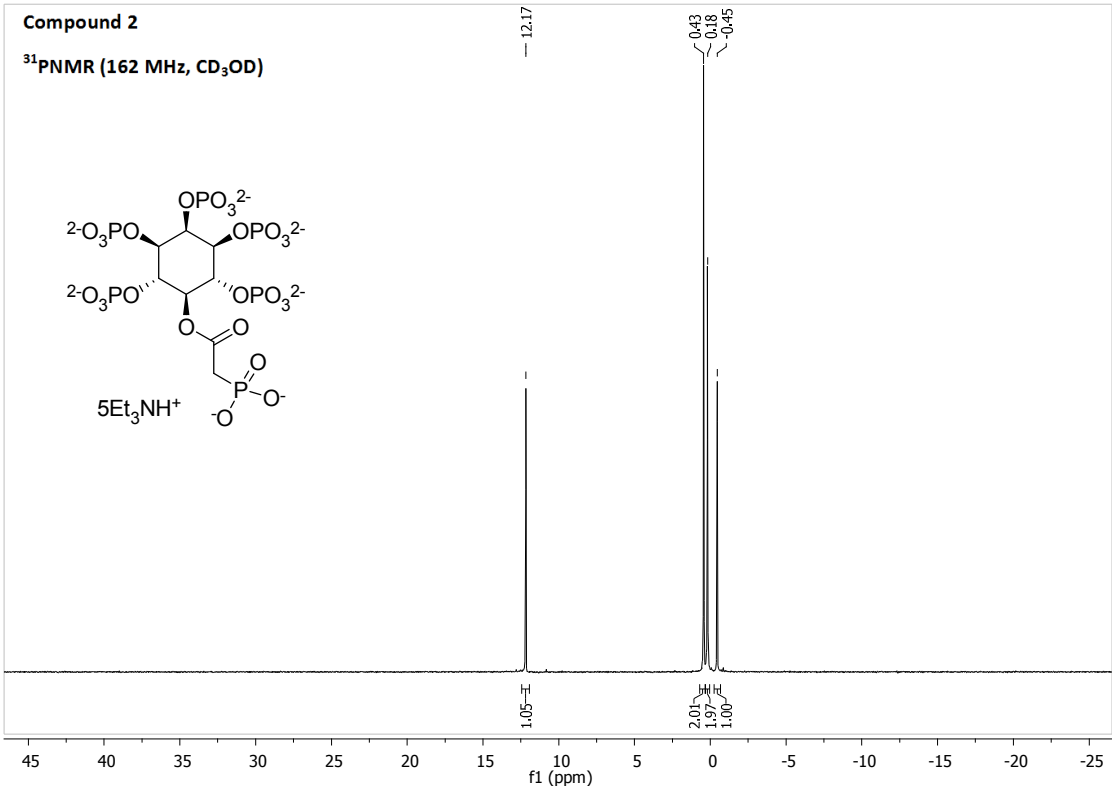
***myo*-inositol 1,2,3,4,6-pentakisphosphate-5-phosphorylacetate (2)**

To a solution of **11** (129 mg, 72.3 μmol) in MeOH (15 mL) and deionised water (2 mL) was added palladium hydroxide on activated charcoal (Fluka, 20%, 50% water, 20 mg). The suspension was stirred vigorously under an atmosphere of hydrogen (balloon) for 16 h. The catalyst was removed by filtration through a PTFE syringe filter to give a colourless solution, which was neutralised with aqueous TEAB (1 M, 1 mL) and then concentrated under reduced pressure. A ^{1}H NMR spectrum in D_2O of the residue at this stage showed no residual aromatic signals, indicating that hydrogenolysis was complete. The residue was purified by ion-exchange chromatography on Q Sepharose Fast Flow resin eluting with a gradient of aqueous TEAB (0 to 2.0 moldm^{-3}) to give the triethylammonium salt of **2** (89 mg, 66 μmol, 92%); ^{1}H NMR (D_2O, 500 MHz, HDO ref at 4.79) δ 1.27 (~48 H, t, J 7.4 Hz, CH_3 of TEA^+), 2.97 (2 H, d, $^{2}J_{HP}$ 20.9 Hz, CH_2P), 3.18 (~31 H, q, J 7.4 Hz, CH_2 of TEA^+), 4.30 (2 H, broad t, J 9.7 Hz, H-1 and H-3), 4.57 (2 H, ddd, J 9.6, 9.5, 9.6 Hz, H-4 and H-6), 4.87 (1 H, dt, J 9.7, 2.5 Hz, H-2), 5.14 (1 H, t, J 9.6 Hz, H-5); ^{13}C NMR (D_2O, 100 MHz) δ 8.31 (CH_3 of TEA^+), 37.53 ($^{1}J_{CP}$ 119.6 Hz, CH_2P), 46.70 (CH_2 of TEA^+), 73.49 (with J_{CP} couplings, C-1 and C-3), 73.84 (broad, C-5), 74.71 (J_{CP} 5.8, 5.8 Hz, C-4 and C-6), 75.75 ($^{2}J_{CP}$ 5.8 Hz C-2), 169.65 ($^{2}J_{CP}$ 6.5 Hz, C=O; ^{31}P NMR (CD_3OD, 162 MHz) δ –0.45 (1 P, phosphate P), 0.18 (2 P, phosphate P), 0.44 (2 P, phosphate

P), 12.17 (1 P, phosphonate P); HRMS (*m*/*z*) $[M]^-$ calcd. for $C_8H_{19}O_{25}P_6$; 700.8647; found 700.8620.



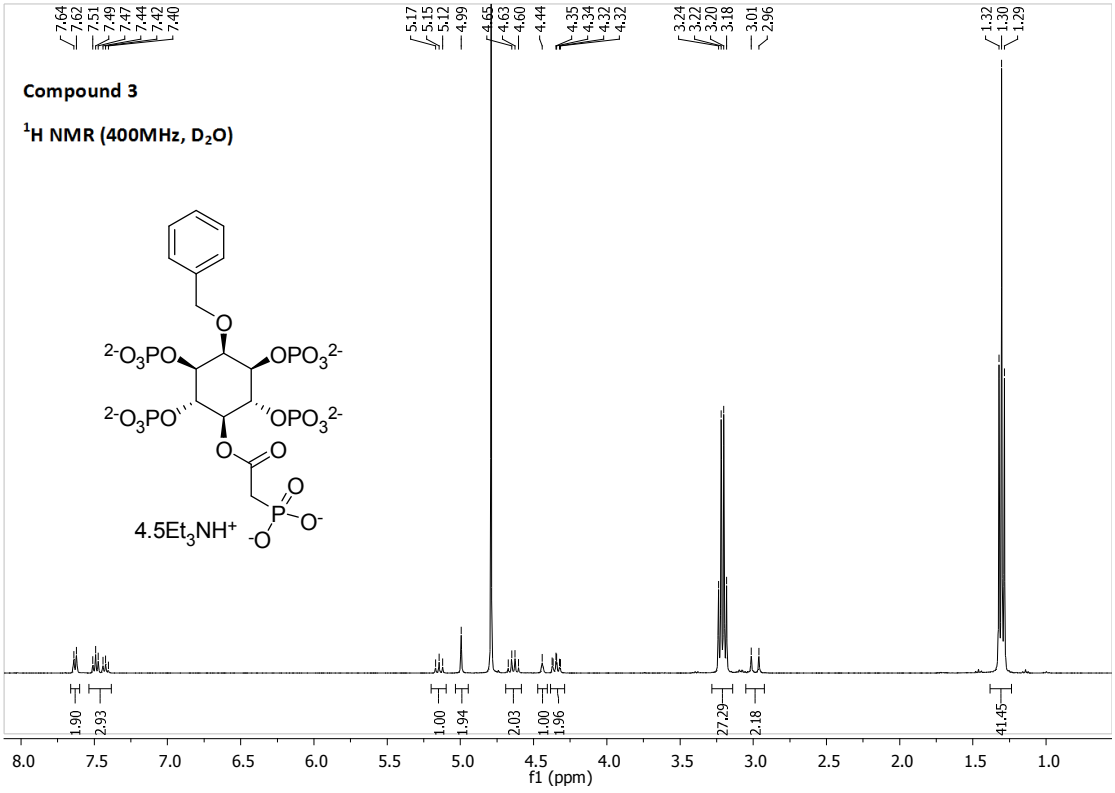


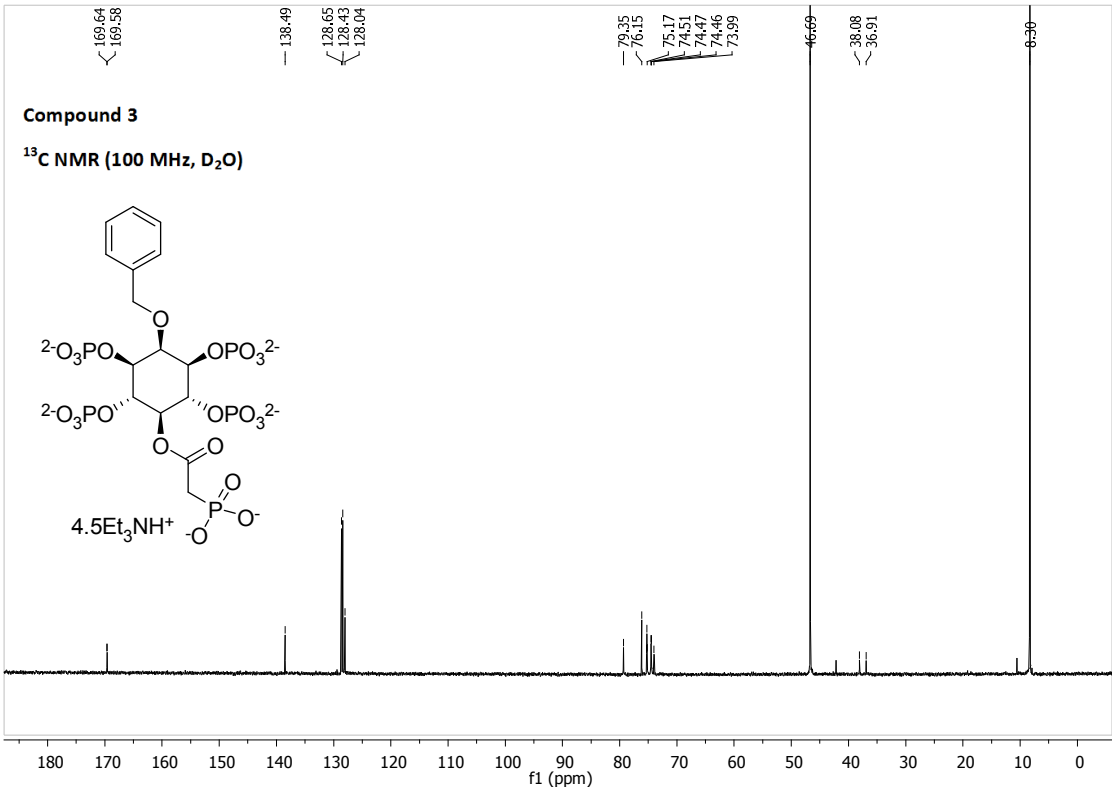


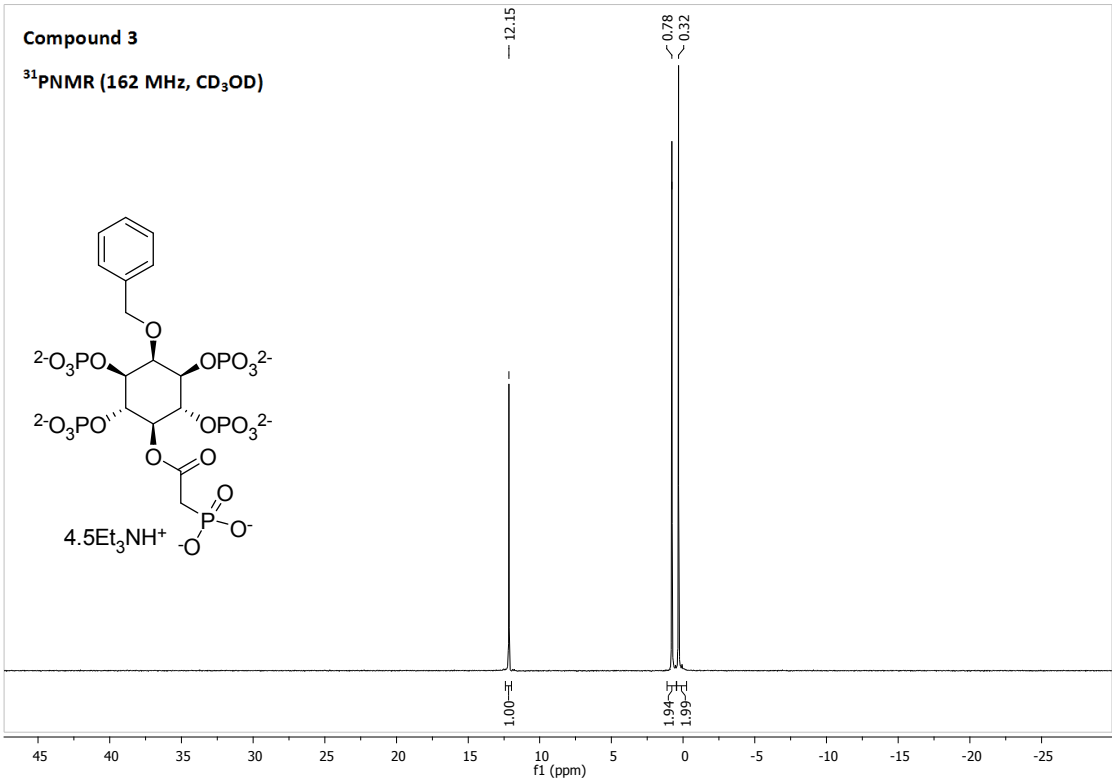
2-*O*-benzyl-*myo*-inositol 1,3,4,6-tetrakisphosphate-5-phosphorylacetate (3)

To a solution of **8** (122 mg, 75 μmol) in MeOH (12 mL) and aqueous TEAB (1.0 moldm^{-3}, pH 7.5, 3 mL) was added palladium hydroxide on activated charcoal (Fluka, 20%, 50% water, 20 mg). The suspension was stirred vigorously under an atmosphere of hydrogen (balloon) for 0.5 h. The catalyst was removed by filtration through a PTFE syringe filter to give a colourless solution, which was concentrated under reduced pressure. A ^{31}P NMR spectrum in CD_3OD of the residue at this stage showed only three peaks and the corresponding ^{1}H NMR spectrum showed that the benzylphosphate esters had been removed, while the benzyl ether was still intact. The residue was re-dissolved in deionised water and lyophilised to give the triethylammonium salt of **3** (73 mg, 59 μmol, 79%); ^{1}H NMR (D_2O, 400 MHz, HDO ref at 4.79) δ 1.30 (~41 H, t, J 7.4 Hz, CH_3 of TEA^+), 2.99 (2 H, d, $^{2}J_{HP}$ 20.9 Hz, CH_2P), 3.21 (~27 H, q, J 7.4 Hz, CH_2 of TEA^+), 4.34 (2 H, ddd, J 9.7 Hz, H-1 and H-3), 4.44 (1 H, br s, H-2), 4.64 (2 H, ddd, J 9.6, 9.5, 9.6 Hz, H-4 and H-6), 4.99 (2 H, s, OCH_2Ph, 5.15 (1 H, t, J 9.6 Hz, H-5);7.40–7.44 (1 H, m, *para*-H of Ph), 7.47–7.51 (2 H, m, *meta*-H of Ph), 7.62–7.64 (2 H, m, *ortho*-H of Ph);^{13}C NMR (D_2O, 100 MHz) δ 8.30 (CH_3 of TEA^+), 37.50 ($^{1}J_{CP}$ 118.1 Hz, CH_2P), 46.69 (CH_2 of TEA^+), 73.99 (broad, C-5), 74.47 (with J_{CP} couplings, C-1 and C-3), 75.23 (J_{CP} 5.6, 5.6 Hz, C-4 and

C-6), 76.15 (O*C*H$_2$Ph), 79.35 (C-2), 128.04 (*para*-C of Ph), 128.43 (Ph), 128.65 (Ph), 138.49 (*ipso*-C of Ph), 169.61 ($^2J_{CP}$ 6.6 Hz, C=O); ^{31}P NMR (CD$_3$OD, 162 MHz) δ 0.32 (2 P, phosphate), 0.78 (2 P, phosphate), 12.15 (1 P, phosphonate); HRMS (*m*/*z*) [M]$^-$ calcd. for $C_{15}H_{24}O_{22}P_5$; 710.9453; found 710.9451.







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